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(54) Title: TRANSMEMBRANE SERINE PROTEASE OVEREXPRESSED IN OVARIAN CARCINOMA AND USES THEREOF (57) Abstract The present invention provides a TADG-12 protein and a DNA fragment encoding such protein. Also provided is a vector/host cell capable of expressing the DNA. The present invention further provided various methods of early detection of associated ovarian and other malignancies, and of interactive therapies for cancer treatment by utilizing the DNA and/or protein disclosed herein.		

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5 **TRANSMEMBRANE SERINE PROTEASE OVEREXPRESSED IN**
 OVARIAN CARCINOMA AND USES THEREOF

BACKGROUND OF THE INVENTION

10 Cross-Reference to Related Application

 This application is a continuation-in-part patent application and claims the benefit of priority under 35 USC §120 of USSN 09/261,416, filed March 3, 1999.

15 Field of the Invention

 The present invention relates generally to the fields of cellular biology and diagnosis of neoplastic disease. More specifically, the present invention relates to a transmembrane serine protease termed Tumor Associated Differentially-Expressed
20 Gene-12 (TADG-12), which is overexpressed in ovarian carcinoma.

Description of the Related Art

 Tumor cells rely on the expression of a concert of proteases to be released from their primary sites and move to
25 distant sites to inflict lethality. This metastatic nature is the result of an aberrant expression pattern of proteases by tumor cells and also by stromal cells surrounding the tumors [1-3]. For most tumors to become metastatic, they must degrade their surrounding extracellular matrix components, degrade basement

membranes to gain access to the bloodstream or lymph system, and repeat this process in reverse fashion to settle in a secondary host site [3-6]. All of these processes rely upon what now appears to be a synchronized protease cascade. In addition, tumor cells
5 use the power of proteases to activate growth and angiogenic factors that allow the tumor to grow progressively [1]. Therefore, much research has been aimed at the identification of tumor-associated proteases and the inhibition of these enzymes for therapeutic means. More importantly, the secreted nature and/or
10 high level expression of many of these proteases allows for their detection at aberrant levels in patient serum, e.g. the prostate-specific antigen (PSA), which allows for early diagnosis of prostate cancer [7].

Proteases have been associated directly with tumor
15 growth, shedding of tumor cells and invasion of target organs. Individual classes of proteases are involved in, but not limited to (1) the digestion of stroma surrounding the initial tumor area, (2) the digestion of the cellular adhesion molecules to allow dissociation of tumor cells; and (3) the invasion of the basement
20 membrane for metastatic growth and the activation of both tumor growth factors and angiogenic factors.

For many forms of cancer, diagnosis and treatment has improved dramatically in the last 10 years. However, the five year survival rate for ovarian cancer remains below 50% due in
25 large part to the vague symptoms which allow for progression of the disease to an advanced stage prior to diagnosis [8]. Although the exploitation of the CA125 antigen has been useful as a marker for monitoring recurrence of ovarian cancer, it has not proven to be an ideal marker for early diagnosis. Therefore, new markers

that may be secreted or released from cells and which are highly expressed by ovarian tumors could provide a useful tool for the early diagnosis and for therapeutic intervention in patients with ovarian carcinoma.

5 The prior art is deficient in the lack of the complete identification of the proteases overexpressed in carcinoma, therefore, deficient in the lack of a tumor marker useful as an indicator of early disease, particularly for ovarian cancers. Specifically, TADG-12, a transmembrane serine protease, has not
10 been previously identified in either nucleic acid or protein form. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

15

 The present invention discloses TADG-12, a new member of the Tumor Associated Differentially-Expressed Gene (TADG) family, and a variant splicing form of TADG-12 (TADG-12V) that could lead to a truncated protein product. TADG-12 is a
20 transmembrane serine protease overexpressed in ovarian carcinoma. The entire cDNA of TADG-12 has been identified (SEQ ID No. 1). This sequence encodes a putative protein of 454 amino acids (SEQ ID No. 2) which includes a potential transmembrane domain, an LDL receptor like domain, a scavenger receptor
25 cysteine rich domain, and a serine protease domain. These features imply that TADG-12 is expressed at the cell surface, and it may be used as a molecular target for therapy or a diagnostic marker.

In one embodiment of the present invention, there is provided a DNA fragment encoding a TADG-12 protein selected from the group consisting of: (a) an isolated DNA fragment which encodes a TADG-12 protein; (b) an isolated DNA fragment which hybridizes to isolated DNA fragment of (a) above and which encodes a TADG-12 protein; and (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Specifically, the DNA fragment has a sequence shown in SEQ ID No. 1 or SEQ ID No. 3.

In another embodiment of the present invention, there is provided a vector/host cell capable of expressing the DNA of the present invention.

In yet another embodiment of the present invention, there is provided an isolated and purified TADG-12 protein encoded by DNA selected from the group consisting of: (a) isolated DNA which encodes a TADG-12 protein; (b) isolated DNA which hybridizes to isolated DNA of (a) above and which encodes a TADG-12 protein; and (c) isolated DNA differing from the isolated DNAs of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Specifically, the TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

In still yet another embodiment of the present invention, there is provided a method for detecting expression of a TADG-12 protein, comprising the steps of: (a) contacting mRNA obtained from the cell with the labeled hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

The present invention further provides methods for diagnosing a cancer or other malignant hyperplasia by detecting the TADG-12 protein or mRNA disclosed herein.

5 In still another embodiment of the present invention, there is provided a method of inhibiting expression of endogenous TADG-12 mRNA in a cell by introducing a vector into the cell, wherein the vector comprises a DNA fragment of TADG-12 in opposite orientation operably linked to elements necessary for expression.

10 In still yet another embodiment of the present invention, there is provided a method of inhibiting expression of a TADG-12 protein in a cell by introducing an antibody directed against a TADG-12 protein or fragment thereof.

15 In still yet another embodiment of the present invention, there is provided a method of targeted therapy by administering a compound having a targeting moiety specific for a TADG-12 protein and a therapeutic moiety. Specifically, the TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

20 The present invention still further provides a method of vaccinating an individual against TADG-12 by inoculating the individual with a TADG-12 protein or fragment thereof. Specifically, the TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4. The TADG-12 fragment
25 includes the truncated form of TADG-12V peptide having a sequence shown in SEQ ID No. 8, and a 9-residue up to 12-residue fragment of TADG-12 protein.

In yet another embodiment of the present invention, there is provided an immunogenic composition, comprising an

immunogenic fragment of a TADG-12 protein and an appropriate adjuvant. The TADG-12 fragment includes the truncated form of TADG-12V peptide having a sequence shown in SEQ ID No. 8, and a 9-residue up to 12-residue fragment of TADG-12 protein.

5. Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

10 BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail,
15 more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the
20 invention and therefore are not to be considered limiting in their scope.

Figure 1A shows that the expected PCR product of approximately 180 bp and the unexpected PCR product of approximately 300 bp using the redundant serine protease
25 primers were not amplified from normal ovary cDNA (Lane 1) but were found in abundance from ovarian tumor cDNA (Lane 2). The primer sequences for the PCR reactions are indicated by horizontal arrows. Figure 1B shows that TADG-12 was subcloned from the 180 bp band while the larger 300 bp band was designated TADG-

12V. The sequences were found to overlap for 180 bp (SEQ ID No. 5 for nucleotide sequence, SEQ ID No. 6 for deduced amino acid sequence) with the 300 bp TADG-12V (SEQ ID No. 7 for nucleotide sequence, SEQ ID No. 8 for deduced amino acid sequence) having an additional insert of 133 bases. This insertion (vertical arrow) leads to a frame shift, which causes the TADG-12V transcript to potentially produce a truncated form of TADG-12 with a variant amino acid sequence.

Figure 2 shows that Northern blot analysis for TADG-12 revealed three transcripts of 2.4, 1.6 and 0.7 kilobases. These transcripts were found at significant levels in ovarian tumors and cancer cell lines, but the transcripts were found only at low levels in normal ovary.

Figure 3 shows an RNA dot blot (CLONTECH) probed for TADG-12. The transcript was detectable (at background levels) in all 50 of the human tissues represented with the greatest abundance of transcript in the heart. Putamen, amygdala, kidney, liver, small intestine, skeletal muscle, and adrenal gland were also found to have intermediate levels of TADG-12 transcript.

Figure 4 shows the entire cDNA sequence for TADG-12 (SEQ ID No. 1) with its predicted open reading frame of 454 amino acids (SEQ ID No. 2). Within the nucleotide sequence, the Kozak's consensus sequence for the initiation of translation and the poly-adenylation signal are underlined. In the protein sequence, a potential transmembrane domain is boxed. The LDLR-A domain is underlined with a solid line. The SRCR domain is underlined with a broken line. The residues of the catalytic triad of the serine protease domain are circled, and the beginning of the

catalytic domain is marked with an arrow designated as a potential proteolytic cleavage site. The * represents the stop codon that terminates translation.

5 **Figure 5A** shows the 35 amino acid LDLR-A domain of TADG-12 (SEQ ID No. 13) aligned with other LDLR-A motifs from the serine protease TMPRSS2 (U75329, SEQ ID No. 14), the complement subunit C8 (P07358, SEQ ID No. 9), two LDLR-A domains of the glycoprotein GP300 (P98164, SEQ ID Nos. 11-12), and the serine protease matriptase (AF118224, SEQ ID No. 10).
10 TADG-12 has its highest similarity with the other serine proteases for which it is 54% similar to TMPRSS2 and 53% similar to matriptase. The highly conserved cysteine residues are shown in bold type. **Figure 5B** shows the SRCR domain of TADG-12 (SEQ ID No. 17) aligned with other domain family members including the
15 human macrophage scavenger receptor (P21757, SEQ ID No. 16), human enterokinase (P98073, SEQ ID No. 19), bovine enterokinase (P21758, SEQ ID No. 15), and the serine protease TMPRSS2 (SEQ ID No. 18). Again, TADG-12 shows its highest similarity within this region to the protease TMPRSS2 at 43%. **Figure 5C** shows the
20 protease domain of TADG-12 (SEQ ID No. 23) in alignment with other human serine proteases including protease M (U62801, SEQ ID No. 20), trypsinogen I (P07477, SEQ ID No. 21), plasma kallikrein (P03952, SEQ ID No. 22), hepsin (P05981, SEQ ID No. 25), and TMPRSS2 (SEQ ID No. 24). Cons represents the consensus
25 sequence for each alignment.

Figure 6 shows semi-quantitative PCR analysis that was performed for TADG-12 (upper panel) and TADG-12V (lower panel). The amplification of TADG-12 or TADG-12V was performed in parallel with PCR amplification of β -tubulin product

as an internal control. The TADG-12 transcript was found to be overexpressed in 41 of 55 carcinomas. The TADG-12V transcript was found to be overexpressed in 8 of 22 carcinomas examined. Note that the samples in the upper panel are not necessarily the same as the samples in the lower panel.

Figure 7 shows immunohistochemical staining of normal ovary and ovarian tumors which were performed using a polyclonal rabbit antibody developed to a TADG-12 specific peptide. No significant staining was detected in normal ovary (**Figure 7A**). Strong positive staining was observed in 22 of 29 carcinomas examined. **Figures 7B** and **7C** represent a serous and mucinous carcinoma, respectively. Both show diffuse staining throughout the cytoplasm of tumor cells while stromal cells remain relatively unstained.

Figure 8 is a model to demonstrate the progression of TADG-12 within a cellular context. In normal circumstances, the TADG-12 transcript is appropriately spliced and the resulting protein is capable of being expressed at the cell surface where the protease may be cleaved to an active form. The role of the remaining ligand binding domains has not yet been determined, but one can envision their potential to bind other molecules for activation, internalization or both. The TADG-12V transcript, which occurs in some tumors, may be the result of mutation and/or poor mRNA processing may be capable of producing a truncated form of TADG-12 that does not have a functional protease domain. In addition, this truncated product may present a novel epitope at the surface of tumor cells.

DETAILED DESCRIPTION OF THE INVENTION

To examine the serine proteases expressed by ovarian cancers, a PCR based differential display technique was employed
5 utilizing redundant PCR primers designed to the most highly conserved amino acids in these proteins [9]. As a result, a novel cell-surface, multi-domain serine protease, named Tumor Associated Differentially-expressed Gene-12 (TADG-12) was identified. TADG-12 appears to be overexpressed in many ovarian
10 tumors. The extracellular nature of TADG-12 may render tumors susceptible to detection via a TADG-12 specific assay. In addition, a splicing variant of TADG-12, named TADG-12V, was detected at elevated levels in 35% of the tumors that were examined. TADG-12V encodes a truncated form of TADG-12 with an altered amino
15 acid sequence that may be a unique tumor specific target for future therapeutic approaches.

The TADG-12 cDNA is 2413 base pairs long (SEQ ID No. 1) encoding a 454 amino acid protein (SEQ ID No. 2). A variant form, TADG-12V (SEQ ID No. 3), encodes a 294 amino acid protein
20 (SEQ ID No. 4). The availability of the TADG-12 and/or TADG-12V gene opens the way for a number studies that can lead to various applications. For example, the TADG-12 and/or TADG-12V gene can be used as a diagnostic or therapeutic target in ovarian carcinoma and other carcinomas including breast, prostate, lung
25 and colon.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Maniatis,

Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. 5 (1985)]; "Transcription and Translation" [B.D. Hames & S.J. Higgins eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

Therefore, if appearing herein, the following terms
10 shall have the definitions set out below.

As used herein, the term "cDNA" shall refer to the DNA copy of the mRNA transcript of a gene.

As used herein, the term "derived amino acid sequence" shall mean the amino acid sequence determined by
15 reading the triplet sequence of nucleotide bases in the cDNA.

As used herein the term "screening a library" shall refer to the process of using a labeled probe to check whether, under the appropriate conditions, there is a sequence complementary to the probe present in a particular DNA library.
20 In addition, "screening a library" could be performed by PCR.

As used herein, the term "PCR" refers to the polymerase chain reaction that is the subject of U.S. Patent Nos. 4,683,195 and 4,683,202 to Mullis, as well as other improvements now known in the art.

25 The amino acid described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property of immunoglobulin-binding is retained by the polypeptide. NH₂ refers to the free amino group present at

the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J Biol. Chem.*, 243:3552-59 (1969), abbreviations for amino acid residues are
5 known in the art.

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted
10 that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA
15 replication *in vivo*; i.e., capable of replication under its own control.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

20 A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms.
25 Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure herein according to the normal convention of giving only the sequence in

the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

5 A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop
10 codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence
15 will usually be located 3' to the coding sequence.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

20 A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site
25 and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for

the binding of RNA polymerase. Eukaryotic promoters often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

5 An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into
10 mRNA, which is then translated into the protein encoded by the coding sequence.

 A "signal sequence" can be included near the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the
15 polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

20 The term "oligonucleotide", as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

25 The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which

is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be "substantially" complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a non-complementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementary with the sequence or hybridize therewith and thereby form the template for the synthesis of the extension product.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the

cell. The transforming DNA may or may not be integrated (covalently linked) into the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid.

5 With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones
10 comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

15 Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90% or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the
20 sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning,
25 Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

A "heterologous" region of the DNA construct is an identifiable segment of DNA within a larger DNA molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the

gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. In another example, coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where
5 the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

The labels most commonly employed for these studies
10 are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others. A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting
15 material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate.

Proteins can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred
20 isotope may be selected from ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re .

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques.
25 The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, β -glucuronidase, β -D-glucosidase, β -D-

galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090, 3,850,752, and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

5 A particular assay system developed and utilized in the art is known as a receptor assay. In a receptor assay, the material to be assayed is appropriately labeled and then certain cellular test colonies are inoculated with a quantity of both the label after which binding studies are conducted to determine the
10 extent to which the labeled material binds to the cell receptors. In this way, differences in affinity between materials can be ascertained.

 An assay useful in the art is known as a "cis/trans" assay. Briefly, this assay employs two genetic constructs, one of
15 which is typically a plasmid that continually expresses a particular receptor of interest when transfected into an appropriate cell line, and the second of which is a plasmid that expresses a reporter such as luciferase, under the control of a receptor/ligand complex. Thus, for example, if it is desired to evaluate a compound as a
20 ligand for a particular receptor, one of the plasmids would be a construct that results in expression of the receptor in the chosen cell line, while the second plasmid would possess a promoter linked to the luciferase gene in which the response element to the particular receptor is inserted. If the compound under test is an
25 agonist for the receptor, the ligand will complex with the receptor, and the resulting complex will bind the response element and initiate transcription of the luciferase gene. The resulting chemiluminescence is then measured photometrically, and dose response curves are obtained and compared to those of known

ligands. The foregoing protocol is described in detail in U.S. Patent No. 4,981,784.

As used herein, the term "host" is meant to include not only prokaryotes but also eukaryotes such as yeast, plant and animal cells. A recombinant DNA molecule or gene which encodes a human TADG-12 protein of the present invention can be used to transform a host using any of the techniques commonly known to those of ordinary skill in the art. Especially preferred is the use of a vector containing coding sequences for the gene which encodes a human TADG-12 protein of the present invention for purposes of prokaryote transformation. Prokaryotic hosts may include *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. Eukaryotic hosts include yeasts such as *Pichia pastoris*, mammalian cells and insect cells.

In general, expression vectors containing promoter sequences which facilitate the efficient transcription of the inserted DNA fragment are used in connection with the host. The expression vector typically contains an origin of replication, promoter(s), terminator(s), as well as specific genes which are capable of providing phenotypic selection in transformed cells. The transformed hosts can be fermented and cultured according to means known in the art to achieve optimal cell growth.

The invention includes a substantially pure DNA encoding a TADG-12 protein, a strand of which DNA will hybridize at high stringency to a probe containing a sequence of at least 15 consecutive nucleotides of the sequence shown in SEQ ID No. 1 or SEQ ID No. 3. The protein encoded by the DNA of this invention may share at least 80% sequence identity (preferably 85%, more preferably 90%, and most preferably 95%) with the amino acids

listed in SEQ ID No. 2 or SEQ ID No. 4. More preferably, the DNA includes the coding sequence of the nucleotides of Figure 4 (SEQ ID No. 1), or a degenerate variant of such a sequence.

5 The probe to which the DNA of the invention hybridizes preferably consists of a sequence of at least 20 consecutive nucleotides, more preferably 40 nucleotides, even more preferably 50 nucleotides, and most preferably 100 nucleotides or more (up to 100%) of the coding sequence of the nucleotides listed in Figure 4 (SEQ ID No. 1) or the complement
10 thereof. Such a probe is useful for detecting expression of TADG-12 in a human cell by a method including the steps of (a) contacting mRNA obtained from the cell with the labeled hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

15 This invention also includes a substantially pure DNA containing a sequence of at least 15 consecutive nucleotides (preferably 20, more preferably 30, even more preferably 50, and most preferably all) of the region from nucleotides 1 to 2413 of the nucleotides listed in SEQ ID No. 1, or of the region from
20 nucleotides 1 to 2544 of the nucleotides listed in SEQ ID No. 3. The present invention also comprises antisense oligonucleotides directed against this novel DNA. Given the teachings of the present invention, a person having ordinary skill in this art would readily be able to develop antisense oligonucleotides directed
25 against this DNA.

By "high stringency" is meant DNA hybridization and wash conditions characterized by high temperature and low salt concentration, e.g., wash conditions of 65°C at a salt concentration of approximately 0.1 x SSC, or the functional equivalent thereof.

For example, high stringency conditions may include hybridization at about 42°C in the presence of about 50% formamide; a first wash at about 65°C with about 2 x SSC containing 1% SDS; followed by a second wash at about 65°C with about 0.1 x SSC.

5 By "substantially pure DNA" is meant DNA that is not part of a milieu in which the DNA naturally occurs, by virtue of separation (partial or total purification) of some or all of the molecules of that milieu, or by virtue of alteration of sequences that flank the claimed DNA. The term therefore includes, for
10 example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by polymerase chain reaction (PCR) or restriction
15 endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence, e.g., a fusion protein. Also included is a recombinant DNA which includes a portion of the nucleotides shown in SEQ ID No. 3 which encodes an
20 alternative splice variant of TADG-12 (TADG-12V).

 The DNA may have at least about 70% sequence identity to the coding sequence of the nucleotides listed in SEQ ID No. 1 or SEQ ID No. 3, preferably at least 75% (e.g. at least 80%); and most preferably at least 90%. The identity between two
25 sequences is a direct function of the number of matching or identical positions. When a subunit position in both of the two sequences is occupied by the same monomeric subunit, e.g., if a given position is occupied by an adenine in each of two DNA molecules, then they are identical at that position. For example, if

7 positions in a sequence 10 nucleotides in length are identical to the corresponding positions in a second 10-nucleotide sequence, then the two sequences have 70% sequence identity. The length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 100 nucleotides. Sequence identity is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 10 1710 University Avenue, Madison, WI 53705).

The present invention comprises a vector comprising a DNA sequence which encodes a human TADG-12 protein and the vector is capable of replication in a host which comprises, in operable linkage: a) an origin of replication; b) a promoter; and c) 15 a DNA sequence coding for said protein. Preferably, the vector of the present invention contains a portion of the DNA sequence shown in SEQ ID No. 1 or SEQ ID No. 3. A "vector" may be defined as a replicable nucleic acid construct, e.g., a plasmid or viral nucleic acid. Vectors may be used to amplify and/or express 20 nucleic acid encoding a TADG-12 protein. An expression vector is a replicable construct in which a nucleic acid sequence encoding a polypeptide is operably linked to suitable control sequences capable of effecting expression of the polypeptide in a cell. The need for such control sequences will vary depending upon the cell 25 selected and the transformation method chosen. Generally, control sequences include a transcriptional promoter and/or enhancer, suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation. Methods which are well known to those skilled in the art can be used to

construct expression vectors containing appropriate transcriptional and translational control signals. See for example, the techniques described in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual* (2nd Ed.), Cold Spring Harbor Press, N.Y. A gene and its transcription control sequences are defined as being "operably linked" if the transcription control sequences effectively control the transcription of the gene. Vectors of the invention include, but are not limited to, plasmid vectors and viral vectors. Preferred viral vectors of the invention are those derived from retroviruses, adenovirus, adeno-associated virus, SV40 virus, or herpes viruses.

By a "substantially pure protein" is meant a protein which has been separated from at least some of those components which naturally accompany it. Typically, the protein is substantially pure when it is at least 60%, by weight, free from the proteins and other naturally-occurring organic molecules with which it is naturally associated *in vivo*. Preferably, the purity of the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight. A substantially pure TADG-12 protein may be obtained, for example, by extraction from a natural source; by expression of a recombinant nucleic acid encoding an TADG-12 polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography such as immunoaffinity chromatography using an antibody specific for TADG-12, polyacrylamide gel electrophoresis, or HPLC analysis. A protein is substantially free of naturally associated components when it is separated from at least some of those contaminants which accompany it in its natural state. Thus, a protein which is

chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be, by definition, substantially free from its naturally associated components. Accordingly, substantially pure proteins include
5 eukaryotic proteins synthesized in *E. coli*, other prokaryotes, or any other organism in which they do not naturally occur.

In addition to substantially full-length proteins, the invention also includes fragments (e.g., antigenic fragments) of the TADG-12 protein. As used herein, "fragment," as applied to a
10 polypeptide, will ordinarily be at least 10 residues, more typically at least 20 residues, and preferably at least 30 (e.g., 50) residues in length, but less than the entire, intact sequence. Fragments of the TADG-12 protein can be generated by methods known to those skilled in the art, e.g., by enzymatic digestion of naturally
15 occurring or recombinant TADG-12 protein, by recombinant DNA techniques using an expression vector that encodes a defined fragment of TADG-12, or by chemical synthesis. The ability of a candidate fragment to exhibit a characteristic of TADG-12 (e.g., binding to an antibody specific for TADG-12) can be assessed by
20 methods described herein. Purified TADG-12 or antigenic fragments of TADG-12 can be used to generate new antibodies or to test existing antibodies (e.g., as positive controls in a diagnostic assay) by employing standard protocols known to those skilled in the art. Included in this invention are polyclonal antisera
25 generated by using TADG-12 or a fragment of TADG-12 as the immunogen in, e.g., rabbits. Standard protocols for monoclonal and polyclonal antibody production known to those skilled in this art are employed. The monoclonal antibodies generated by this procedure can be screened for the ability to identify recombinant

TADG-12 cDNA clones, and to distinguish them from known cDNA clones.

Further included in this invention are TADG-12 proteins which are encoded at least in part by portions of SEQ ID No. 1 or SEQ ID No. 3, e.g., products of alternative mRNA splicing or alternative protein processing events, or in which a section of TADG-12 sequence has been deleted. The fragment, or the intact TADG-12 polypeptide, may be covalently linked to another polypeptide, e.g. which acts as a label, a ligand or a means to increase antigenicity.

The invention also includes a polyclonal or monoclonal antibody which specifically binds to TADG-12. The invention encompasses not only an intact monoclonal antibody, but also an immunologically-active antibody fragment, e.g., a Fab or (Fab)₂ fragment; an engineered single chain Fv molecule; or a chimeric molecule, e.g., an antibody which contains the binding specificity of one antibody, e.g., of murine origin, and the remaining portions of another antibody, e.g., of human origin.

In one embodiment, the antibody, or a fragment thereof, may be linked to a toxin or to a detectable label, e.g. a radioactive label, non-radioactive isotopic label, fluorescent label, chemiluminescent label, paramagnetic label, enzyme label, or colorimetric label. Examples of suitable toxins include diphtheria toxin, *Pseudomonas* exotoxin A, ricin, and cholera toxin. Examples of suitable enzyme labels include malate hydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease,

urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, acetylcholinesterase, etc. Examples of suitable radioisotopic labels include ^3H , ^{125}I , ^{131}I , ^{32}P , ^{35}S , ^{14}C , etc.

Paramagnetic isotopes for purposes of *in vivo* diagnosis can also be used according to the methods of this invention. There are numerous examples of elements that are useful in magnetic resonance imaging. For discussions on *in vivo* nuclear magnetic resonance imaging, see, for example, Schaefer et al., (1989) *JACC* 14, 472-480; Shreve et al., (1986) *Magn. Reson. Med.* 3, 336-340; Wolf, G. L., (1984) *Physiol. Chem. Phys. Med. NMR* 16, 93-95; Wesbey et al., (1984) *Physiol. Chem. Phys. Med. NMR* 16, 145-155; Runge et al., (1984) *Invest. Radiol.* 19, 408-415. Examples of suitable fluorescent labels include a fluorescein label, an isothiocyalate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an ophthaldehyde label, a fluorescamine label, etc. Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, an aequorin label, etc.

Those of ordinary skill in the art will know of other suitable labels which may be employed in accordance with the present invention. The binding of these labels to antibodies or fragments thereof can be accomplished using standard techniques commonly known to those of ordinary skill in the art. Typical techniques are described by Kennedy et al., (1976) *Clin. Chim. Acta* 70, 1-31; and Schurs et al., (1977) *Clin. Chim. Acta* 81, 1-40. Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide

method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method. All of these methods are incorporated by reference herein.

Also within the invention is a method of detecting
5 TADG-12 protein in a biological sample, which includes the steps of contacting the sample with the labeled antibody, e.g., radioactively tagged antibody specific for TADG-12, and determining whether the antibody binds to a component of the sample.

10 As described herein, the invention provides a number of diagnostic advantages and uses. For example, the TADG-12 protein disclosed in the present invention is useful in diagnosing cancer in different tissues since this protein is highly overexpressed in tumor cells. Antibodies (or antigen-binding
15 fragments thereof) which bind to an epitope specific for TADG-12, are useful in a method of detecting TADG-12 protein in a biological sample for diagnosis of cancerous or neoplastic transformation. This method includes the steps of obtaining a biological sample (e.g., cells, blood, plasma, tissue, etc.) from a patient suspected of
20 having cancer, contacting the sample with a labeled antibody (e.g., radioactively tagged antibody) specific for TADG-12, and detecting the TADG-12 protein using standard immunoassay techniques such as an ELISA. Antibody binding to the biological sample indicates that the sample contains a component which specifically
25 binds to an epitope within TADG-12.

Likewise, a standard Northern blot assay can be used to ascertain the relative amounts of TADG-12 mRNA in a cell or tissue obtained from a patient suspected of having cancer, in accordance with conventional Northern hybridization techniques

known to those of ordinary skill in the art. This Northern assay uses a hybridization probe, e.g. radiolabelled TADG-12 cDNA, either containing the full-length, single stranded DNA having a sequence complementary to SEQ ID No. 1 or SEQ ID No. 3, or a
5 fragment of that DNA sequence at least 20 (preferably at least 30, more preferably at least 50, and most preferably at least 100 consecutive nucleotides in length). The DNA hybridization probe can be labeled by any of the many different methods known to those skilled in this art.

10 Antibodies to the TADG-12 protein can be used in an immunoassay to detect increased levels of TADG-12 protein expression in tissues suspected of neoplastic transformation. These same uses can be achieved with Northern blot assays and analyses.

15 The present invention is directed to DNA fragment encoding a TADG-12 protein selected from the group consisting of:
(a) an isolated DNA fragment which encodes a TADG-12 protein;
(b) an isolated DNA fragment which hybridizes to isolated DNA fragment of (a) above and which encodes a TADG-12 protein; and
20 (c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Preferably, the DNA has the sequence shown in SEQ ID No. 1 or SEQ ID No. 3. More preferably, the DNA encodes a TADG-
25 12 protein having the amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

The present invention is also directed to a vector and/or a host cell capable of expressing the DNA of the present invention. Preferably, the vector contains DNA encoding a TADG-

12 protein having the amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4. Representative host cells include bacterial cells, yeast cells, mammalian cells and insect cells.

The present invention is also directed to an isolated
5 and purified TADG-12 protein coded for by DNA selected from the group consisting of: (a) isolated DNA which encodes a TADG-12 protein; (b) isolated DNA which hybridizes to isolated DNA of (a) above and which encodes a TADG-12 protein; and (c) isolated DNA
10 differing from the isolated DNAs of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein. Preferably, the isolated and purified TADG-12 protein has the amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4.

The present invention is also directed to a method of
15 detecting expression of the TADG-12 protein described herein, comprising the steps of: (a) contacting mRNA obtained from the cell with the labeled hybridization probe; and (b) detecting hybridization of the probe with the mRNA.

A number of potential applications are possible for the
20 TADG-12 gene and gene product including the truncated product TADG-12V.

In one embodiment of the present invention, there is provided a method for diagnosing a cancer by detecting a TADG-12 protein in a biological sample, wherein the presence or absence
25 of a TADG-12 protein indicates the presence or absence of a cancer. Preferably, the biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells. Still preferably, the detection of TADG-12 protein is by means selected

from the group consisting of Northern blot, Western blot, PCR, dot blot, ELIZA sandwich assay, radioimmunoassay, DNA array chips and flow cytometry. Such method is used for detecting an ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer
5 and other cancers in which TADG-12 is overexpressed.

In another embodiment of the present invention, there is provided a method for detecting malignant hyperplasia by detecting a TADG-12 protein or TADG-12 mRNA in a biological sample. Further by comprising the TADG-12 protein or TADG-12
10 mRNA to reference information, a diagnosis or a treatment can be provided. Preferably, PCR amplification is used for detecting TADG-12 mRNA, wherein the primers utilized are selected from the group consisting of SEQ ID Nos. 28-31. Still preferably, detection of a TADG-12 protein is by immunoaffinity to an
15 antibody directed against a TADG-12 protein.

In still another embodiment of the present invention, there is provided a method of inhibiting expression of endogenous TADG-12 mRNA in a cell by introducing a vector comprising a DNA fragment of TADG-12 in opposite orientation operably linked to
20 elements necessary for expression. As a result, the vector produces TADG-12 antisense mRNA in the cell, which hybridizes to endogenous TADG-12 mRNA, thereby inhibiting expression of endogenous TADG-12 mRNA.

In still yet another embodiment of the present
25 invention, there is provided a method of inhibiting expression of a TADG-12 protein by introducing an antibody directed against a TADG-12 protein or fragment thereof. As a result, the binding of the antibody to the TADG-12 protein or fragment thereof inhibits the expression of the TADG-12 protein.

TADG-12 gene products including the truncated form can be used for targeted therapy. Specifically, a compound having a targeting moiety specific for a TADG-12 protein and a therapeutic moiety is administered to an individual in need of such treatment. Preferably, the targeting moiety is selected from the group consisting of an antibody directed against a TADG-12 protein and a ligand or ligand binding domain that binds a TADG-12 protein. The TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4. Still preferably, the therapeutic moiety is selected from the group consisting of a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant and a cytotoxic agent. Such method can be used for treating an individual having a disease selected from the group consisting of ovarian cancer, lung cancer, prostate cancer, colon cancer and other cancers in which TADG-12 is overexpressed.

In yet another embodiment of the present invention, there is provided a method of vaccinating, or producing an immune response in, an individual against TADG-12 by inoculating the individual with a TADG-12 protein or fragment thereof. Specifically, the TADG-12 protein or fragment thereof lacks TADG-12 activity, and the inoculation elicits an immune response in the individual, thereby vaccinating the individual against TADG-12. Preferably, the individual has a cancer, is suspected of having a cancer or is at risk of getting a cancer. Still preferably, TADG-12 protein has an amino acid sequence shown in SEQ ID No. 2 or SEQ ID No. 4, while TADG-12 fragment has a sequence shown in SEQ ID No. 8, or is a 9-residue fragment up to a 20-residue fragment. Examples of 9-residue fragment are shown in SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

In still yet another embodiment of the present invention, there is provided an immunogenic composition, comprising an immunogenic fragment of a TADG-12 protein and an appropriate adjuvant. Preferably, the immunogenic fragment of the TADG-12 protein has a sequence shown in SEQ ID No. 8, or is a 9-residue fragment up to a 20-residue fragment. Examples of 9-residue fragment are shown in SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

Tissue collection and storage

Upon patient hysterectomy, bilateral salpingo-oophorectomy, or surgical removal of neoplastic tissue, the specimen is retrieved and placed on ice. The specimen was then taken to the resident pathologist for isolation and identification of specific tissue samples. Finally, the sample was frozen in liquid nitrogen, logged into the laboratory record and stored at -80°C. Additional specimens were frequently obtained from the Cooperative Human Tissue Network (CHTN). These samples were prepared by the CHTN and shipped on dry ice. Upon arrival, these specimens were logged into the laboratory record and stored at -80°C.

EXAMPLE 2

mRNA Extraction and cDNA Synthesis

Sixty-nine ovarian tumors (4 benign tumors, 10 low malignant potential tumors and 55 carcinomas) and 10 normal

ovaries were obtained from surgical specimens and frozen in liquid nitrogen. The human ovarian carcinoma cell lines SW 626 and Caov 3, the human breast carcinoma cell lines MDA-MB-231 and MDA-MB-435S were purchased from the American Type Culture Collection (Rockville, MD). Cells were cultured to sub-confluency in Dulbecco's modified Eagle's medium, supplemented with 10% (v/v) fetal bovine serum and antibiotics.

Extraction of mRNA and cDNA synthesis were carried out by the methods described previously [14-16]. mRNA was isolated by using a RiboSep mRNA isolation kit (Becton Dickinson Labware). In this procedure, poly A+ mRNA was isolated directly from the tissue lysate using the affinity chromatography media oligo(dT) cellulose. cDNA was synthesized with 5.0 µg of mRNA by random hexamer priming using 1st strand cDNA synthesis kit (CLONTECH).

EXAMPLE 3

PCR with Redundant Primers and Cloning of TADG-12 cDNA

Redundant primers, forward 5'-
 20 TGGGTIGTIACIGCIGCICA(CT)TG -3' (SEQ ID No. 26) and reverse 5'-
 A(AG)IA(AG)IGCIATITCITTICC-3' (SEQ ID No. 27), for the
 consensus sequences of amino acids surrounding the catalytic
 triad for serine proteases were used to compare the PCR products
 from normal and carcinoma cDNAs. The appropriate bands were
 25 ligated into Promega T-vector plasmid and the ligation product
 was used to transform JM109 cells (Promega) grown on selection
 media. After selection of individual colonies, they were cultured
 and plasmid DNA was isolated by means of the Wizard miniprep
 DNA purification system (Promega). Nucleotide sequencing was

performed using PRISM Ready Reaction Dye Deoxy terminator cycle sequencing kit (Applied Biosystems). Applied Biosystems Model 373A DNA sequencing system was used for direct cDNA sequence determination.

5 The original TADG-12 subclone was randomly labeled and used as a probe to screen an ovarian tumor cDNA library by standard hybridization techniques [11,15]. The library was constructed in λ ZAP using mRNA isolated from the tumor cells of a stage III/grade III ovarian adenocarcinoma patient. Three
10 overlapping clones were obtained which spanned 2315 nucleotides. The final 99 nucleotides encoding the most 3' sequence including the poly A tail was identified by homology with clones available in the GenBank EST database.

15

EXAMPLE 4

Quantitative PCR

The mRNA overexpression of TADG-12 was determined using a quantitative PCR. Quantitative PCR was performed according to the procedure as previously reported [16].
20 Oligonucleotide primers were used for: TADG-12, forward 5'-GAAACATGTCCTTGCTCTCG-3' (SEQ ID No. 28) and reverse 5'-ACTAACTTCCACAGCCTCCT-3' (SEQ ID No. 29); the variant TADG-12, forward 5'-TCCAGGTGGGTCTAGTTTCC-3' (SEQ ID No. 30), reverse 5'-CTCTTTGGCTTGTACTTGCT-3' (SEQ ID No. 31); β -tubulin, forward
25 5'- CGCATCAACGTGTACTACAA -3' (SEQ ID No. 32) and reverse 5'-TACGAGCTGGTGGACTGAGA -3' (SEQ ID No. 33). β -tubulin was utilized as an internal control. The PCR reaction mixture consists of cDNA derived from 50 ng of mRNA, 5 pmol of sense and antisense primers for both the TADG-12 gene and the β -tubulin

gene, 200 μ mol of dNTPs, 5 μ Ci of α -³²PdCTP and 0.25 unit of Taq DNA polymerase with reaction buffer (Promega) in a final volume of 25 μ l. The target sequences were amplified in parallel with the β -tubulin gene. Thirty cycles of PCR were carried out in a Thermal
5 Cycycler (Perkin-Elmer Cetus). Each cycle of PCR included 30 seconds of denaturation at 94°C, 30 seconds of annealing at 60°C and 30 seconds of extension at 72°C. The PCR products were separated on 2% agarose gels and the radioactivity of each PCR product was determined by using a Phospho Imager (Molecular
10 Dynamics). The present study used the expression ratio (TADG-12/ β -tubulin) as measured by phosphoimager to evaluate gene expression and defined the value at mean + 2SD of normal ovary as the cut-off value to determine overexpression. The student's *t* test was used for comparison of the mean values of normal ovary
15 and tumors.

EXAMPLE 5

Sequencing of TADG-12/TADG-12V

Utilizing a plasmid specific primer near the cloning
20 site, sequencing reactions were carried out using PRISM™ Ready Reaction Dye Deoxy™ terminators (Applied Biosystems cat# 401384) according to the manufacturer's instructions. Residual dye terminators were removed from the completed sequencing reaction using a Centri-sep™ spin column (Princeton Separation
25 cat.# CS-901). An Applied Biosystems Model 373A DNA Sequencing System was available and was used for sequence analysis.

EXAMPLE 6

Antibody Production

Polyclonal rabbit antibodies were generated by immunization of white New Zealand rabbits with a poly-lysine linked multiple antigen peptide derived from the TADG-12 carboxy-terminal protein sequence NH₂-WIHEQMERDLKT-COOH (WIHEQMERDLKT, SEQ ID No. 34). This peptide is present in full length TADG-12, but not TADG-12V. Rabbits were immunized with approximately 100 µg of peptide emulsified in Ribi adjuvant. Subsequent boost immunizations were carried out at 3 and 6 weeks, and rabbit serum was isolated 10 days after the boost inoculations. Sera were tested by dot blot analysis to determine affinity for the TADG-12 specific peptide. Rabbit pre-immune serum was used as a negative control.

15

EXAMPLE 7

Northern Blot Analysis

10 µg of mRNA were loaded onto a 1% formaldehyde-agarose gel, electrophoresed and blotted on a Hybond-N+ nylon membrane (Amersham). ³²P-labeled cDNA probes were made by Prime-a-Gene Labeling System (Promega). The PCR products amplified by the same primers as above were used for probes. The blots were prehybridized for 30 min and hybridized for 60 min at 68°C with ³²P-labeled cDNA probe in ExpressHyb Hybridization Solution (CLONTECH). Control hybridization to determine relative gel loading was performed with the β-tubulin probe.

Normal human tissues; spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocyte, and normal human fetal tissues; brain, lung, liver and kidney (Human Multiple Tissue Northern Blot; CLONTECH) were also examined by
5 same hybridization procedure.

EXAMPLE 8

Immunohistochemistry

Immunohistochemical staining was performed using a
10 Vectastain Elite ABC Kit (Vector). Formalin fixed and paraffin embedded specimens were routinely deparaffinized and processed using microwave heat treatment in 0.01 M sodium citrate buffer (pH 6.0). The specimens were incubated with normal goat serum in a moist chamber for 30 minutes. TADG-12 peptide antibody
15 was allowed to incubate with the specimens in a moisture chamber for 1 hour. Excess antibody was washed away with phosphate buffered saline. After incubation with biotinylated anti-rabbit IgG for 30 minutes, the sections were then incubated with ABC reagent (Vector) for 30 minutes. The final products
20 were visualized using the AEC substrate system (DAKO) and sections were counterstained with hematoxylin before mounting. Negative controls were performed by using normal serum instead of the primary antibody.

25

EXAMPLE 9

Isolation of Catalytic Domain Subclones of TADG-12 and TADG-12 Variant

To identify serine proteases that are expressed in ovarian tumors, redundant PCR primers designed to the conserved

regions of the catalytic triad of these enzymes were employed. A sense primer designed to the region surrounding the conserved histidine and an anti-sense primer designed to the region surrounding the conserved aspartate were used in PCR reactions with either normal ovary or ovarian tumor cDNA as template. In the reaction with ovarian tumor cDNA, a strong product band of the expected size of approximately 180 bp was observed as well as an unexpected PCR product of approximately 300 bp which showed strong expression in some ovarian tumor cDNA's (Figure 1A). Both of these PCR products were subcloned and sequenced. The sequence of the subclones from the 180bp band (SEQ ID No. 5) was found to be homologous to the sequence identified in the larger, unexpected band (SEQ ID No. 7) except that the larger band had an additional insert of 133 nucleotides (Figure 1B). The smaller product of the appropriate size encoded for a protein sequence (SEQ ID No. 6) homologous to other known proteases while the sequence with the insertion (SEQ ID No. 8) encoded for a frame shift from the serine protease catalytic domain and a subsequent premature translational stop codon. TADG-12 variants from four individual tumors were also subcloned and sequenced. It was found that the sequence and insert to be identical. The genomic sequences for these cDNA derived clones were amplified by PCR, examined and found to contain potential AG/GT splice sites that would allow for the variant transcript production.

25

EXAMPLE 10

Northern Blot Analysis of TADG-12 Expression

To examine transcript size and tissue distribution, the catalytic domain subclone was randomly labeled and used to

probe Northern blots representing normal ovarian tissue, ovarian tumors and the cancer cell lines SW626, CAOV3, HeLa, MD-MBA-435S and MD-MBA-231 (Figure 2). Three transcripts of 2.4, 1.6 and 0.7 kilobases were observed. In blots of normal and ovary
5 tumor the smallest transcript size 0.7 kb was lowly expressed in normal ovary while all transcripts (2.4, 1.6 and 0.7 kb) were abundantly present in serous carcinoma. In addition, Northern blots representing the normal human tissues spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood
10 leukocyte, and normal human fetal tissues of brain, lung, liver and kidney were examined. The same three transcripts were found to be expressed weakly in all of these tissues (data not shown). A human β -tubulin specific probe was utilized as a control for relative sample loading. In addition, an RNA dot blot was probed
15 representing 50 human tissues and determined that this clone is weakly expressed in all tissues represented (Figure 3). It was found most prominently in heart, with intermediate levels in putamen, amygdala, kidney, liver, small intestine, skeletal muscle, and adrenal gland.

20

EXAMPLE 11

Sequencing and Characterization of TADG-12

An ovarian tumor cDNA library constructed in λ ZAP was screened by standard hybridization techniques using the
25 catalytic domain subclone as a probe. Two clones that overlapped with the probe were identified and sequenced and found to represent 2316 nucleotides. The 97 nucleotides at the 3' end of the transcript including the poly-adenylation signal and the poly (A) tail were identified by homology with clones available in

GenBank's EST database. This brought the total size of the transcript to 2413 bases (SEQ ID No. 1, Figure 4). Subsequent screening of GenBank's Genomic Database revealed that TADG-12 is homologous to a cosmid from chromosome 17. This cosmid has
5 the accession number AC015555.

The identified cDNA includes an open reading frame that would produce a predicted protein of 454 amino acids (SEQ ID No. 2), named Tumor Associated Differentially-Expressed Gene 12 (TADG-12). The sequence has been submitted to the GenBank
10 database and granted the accession # AF201380. Using homology alignment programs, this protein contains several domains including an amino-terminal cytoplasmic domain, a potential Type II transmembrane domain followed by a low-density lipoprotein receptor-like class A domain (LDLR-A), a scavenger receptor
15 cysteine rich domain (SRCR), and an extracellular serine protease domain.

As predicted by the TMPred program, TADG-12 contains a highly hydrophobic stretch of amino acids that could serve as a potential transmembrane domain, which would retain the amino
20 terminus of the protein within the cytoplasm and expose the ligand binding domains and protease domain to the extracellular space. This general structure is consistent with other known transmembrane proteases including hepsin [17], and TMPRSS2 [18], and TADG-12 is particularly similar in structure to the
25 TMPRSS2 protease.

The LDLR-A domain of TADG-12 is represented by the sequence from amino acid 74 to 108 (SEQ ID No. 13). The LDLR-A domain was originally identified within the LDL Receptor [19] as a series of repeated sequences of approximately 40 amino acids,

which contained 6 invariant cysteine residues and highly conserved aspartate and glutamate residues. Since that initial identification, a host of other genes have been identified which contain motifs homologous to this domain [20]. Several proteases
5 have been identified which contain LDLR-A motifs including matriptase, TMPRSS2 and several complement components. A comparison of TADG-12 with other known LDLR-A domains is shown in Figure 5A. The similarity of these sequences range from 44 to 54% of similar or identical amino acids.

10 In addition to the LDLR-A domain, TADG-12 contains another extracellular ligand binding domain with homology to the group A SRCR family. This family of protein domains typically is defined by the conservation of 6 cysteine residues within a sequence of approximately 100 amino acids [23]. The SRCR
15 domain of TADG-12 is encoded by amino acids 109 to 206 (SEQ ID No. 17), and this domain was aligned with other SRCR domains and found to have between 36 and 43% similarity (Figure 5B). However, TADG-12 only has 4 of the 6 conserved cysteine residues. This is similar to the SRCR domain found in the protease
20 TMPRSS2.

The TADG-12 protein also includes a serine protease domain of the trypsin family of proteases. An alignment of the catalytic domain of TADG-12 with other known proteases is shown in Figure 5C. The similarity among these sequence ranges from 48
25 to 55%, and TADG-12 is most similar to the serine protease TMPRSS2 which also contains a transmembrane domain, LDLR-A domain and an SRCR domain. There is a conserved amino acid motif (RIVGG) downstream from the SRCR domain that is a potential cleavage/activation site common to many serine

proteases of this family [25]. This suggests that TADG-12 is trafficked to the cell surface where the ligand binding domains are capable of interacting with extracellular molecules and the protease domain is potentially activated. TADG-12 also contains
5 conserved cysteine residues (amino acids 208 and 243) which in other proteases form a disulfide bond capable of linking the activated protease to the other extracellular domains.

EXAMPLE 12

10 Quantitative PCR Characterization of the Alternative Transcript

The original TADG-12 subclone was identified as highly expressed in the initial redundant-primer PCR experiment. The TADG-12 variant form (TADG-12V) with the insertion of 133 bp was also easily detected in the initial experiment. To identify
15 the frequency of this expression and whether or not the expression level between normal ovary and ovarian tumors was different, a previously authenticated semi-quantitative PCR technique was employed [16]. The PCR analysis co-amplified a product for β -tubulin with either a product specific to TADG-12 or
20 TADG-12V in the presence of a radiolabelled nucleotide. The products were separated by agarose gel electrophoresis and a phosphoimager was used to quantitate the relative abundance of each PCR product. Examples of these PCR amplification products are shown for both TADG-12 and TADG-12V in Figure 6. Normal
25 expression was defined as the mean ratio of TADG-12 (or TADG-12V) to β -tubulin \pm 2SD as examined in normal ovarian samples. For tumor samples, overexpression was defined as $>2SD$ from the normal TADG-12/ β -tubulin or TADG-12V/ β -tubulin ratio. The results are summarized in Table 1 and Table 2. TADG-12 was

found to be overexpressed in 41 of 55 carcinomas examined while the variant form was present at aberrantly high levels in 8 of 22 carcinomas. As determined by the student's t test, these differences were statistically significant ($p < 0.05$).

5

TABLE 1**Frequency of Overexpression of TADG-12 in Ovarian Carcinoma**

Histology Type	TADG-12 (%)
Normal	0/16 (0%)
LMP-Serous	3/6 (50%)
LMP-Mucinous	0/4 (0%)
Serous Carcinoma	23/29 (79%)
Mucinous Carcinoma	7/12 (58%)
Endometrioid Carcinoma	8/8 (100%)
Clear Cell Carcinoma	3/6 (50%)
Benign Tumors	3/4 (75%)

10 Overexpression = more than two standard deviations above the mean for normal ovary

LMP = low malignant potential tumor

TABLE 2**Frequency of Overexpression of TADG-12V in Ovarian Carcinoma**

Histology Type	TADG-12V (%)
Normal	0/10 (0%)
LMP-Serous	0/5 (0%)
LMP-Mucinous	0/3 (0%)
Serous Carcinoma	4/14 (29%)
Mucinous Carcinoma	3/5 (60%)
Endometrioid Carcinoma	1/3 (33%)
Clear Cell Carcinoma	N/D

Overexpression = more than two standard deviations above
 5 the mean for normal ovary; LMP = low malignant potential tumor

EXAMPLE 13**Immunohistochemical Analysis of TADG-12 in Ovarian Tumor Cells**

10 In order to examine the TADG-12 protein, polyclonal rabbit anti-sera to a peptide located in the carboxy-terminal amino acid sequence was developed. These antibodies were used to examine the expression level of the TADG-12 protein and its localization within normal ovary and ovarian tumor cells by
 15 immuno-localization. No staining was observed in normal ovarian tissues (Figure 7A) while significant staining was observed in 22 of 29 tumors studied. Representative tumor samples are shown in Figures 7B and 7C. It should be noted that TADG-12 is found in a diffuse pattern throughout the cytoplasm indicative of a protein in
 20 a trafficking pathway. TADG-12 is also found at the cell surface in these tumor samples as expected. It should be noted that the

antibody developed and used for immunohistochemical analysis would not detect the TADG-12V truncated protein.

The results of the immunohistochemical staining are summarized in Table 3. 22 of 29 ovarian tumors showed positive staining of TADG-12, whereas normal ovarian surface epithelium showed no expression of the TADG-12 antigen. 8 of 10 serous adenocarcinomas, 8 of 8 mucinous adenocarcinomas, 1 of 2 clear cell carcinomas, and 4 of 6 endometrioid carcinomas showed positive staining.

10

TABLE 3

Case	Stage	Histology	Grade	LN*	TADG12	Prognosis
1		Normal ovary			0-	
2		Normal ovary			0-	
3		Normal ovary			0-	
4		Mucinous B		ND	0-	Alive
5		Mucinous B		ND	1+	Alive
6	1 a	Serous LMP	G1	ND	1+	Alive
7	1 a	Mucinous LMP	G1	ND	1+	Alive
8	1 a	Mucinous CA	G1	ND	1+	Alive
9	1 a	Mucinous CA	G2	ND	1+	Alive
10	1 a	Endometrioid CA	G1	ND	0-	Alive
11	1c	Serous CA	G1	N	1+	Alive
12	1c	Mucinous CA	G1	N	1+	Alive
13	1c	Mucinous CA	G1	N	2+	Alive
14	1c	Clear cell CA	G2	N	0-	Alive
15	1c	Clear cell CA	G2	N	0-	Alive
16	2c	Serous CA	G3	N	2+	Alive
17	3 a	Mucinous CA	G2	N	2+	Alive

18	3 b	Serous CA	G1	ND	1+	Alive
19	3 c	Serous CA	G1	N	0 -	Dead
20	3 c	Serous CA	G3	P	1+	Alive
21	3 c	Serous CA	G2	P	2+	Alive
22	3 c	Serous CA	G1	P	2+	Unknown
23	3 c	Serous CA	G3	ND	2+	Alive
24	3 c	Serous CA	G2	N	0 -	Dead
25	3 c	Mucinous CA	G1	P	2+	Dead
26	3 c	Mucinous CA	G2	ND	1+	Unknown
27	3 c	Mucinous CA	G2	N	1+	Alive
28	3 c	Endometrioid CA	G1	P	1+	Dead
29	3 c	Endometrioid CA	G2	N	0 -	Alive
30	3 c	Endometrioid CA	G2	P	1+	Dead
31	3 c	Endometrioid CA	G3	P	1+	Alive
32	3 c	Clear Cell CA	G3	P	2+	Dead

LN*= Lymph Node: B = Benign; N = Negative; P = Positive;

ND = Not Done

5

EXAMPLE 14

Peptide Ranking

For vaccine or immune stimulation, individual 9-mers to 11-mers of the TADG-12 protein were examined to rank the binding of individual peptides to the top 8 haplotypes in the general population [Parker et al., (1994)]. The computer program used for this analysis can be found at <http://www-bimas.dcert.nih.gov/molbio/hla_bind/>. Table 4 shows the peptide ranking based upon the predicted half-life of each peptide's binding to a particular HLA allele. A larger half-life indicates a

stronger association with that peptide and the particular HLA molecule. The TADG-12 peptides that strongly bind to an HLA allele are putative immunogens, and are used to inoculate an individual against TADG-12.

5

TABLE 4

TADG-12 peptide ranking					
HLA Type				Predicted	SEQ
	& Ranking	Start	Peptide	Dissociation _{1/2}	ID No.
10	HLA A0201				
	1	40	ILSLLPFEV	685.783	35
	2	144	AQLGFPSYV	545.316	36
	3	225	LLSQWPWQA	63.342	37
	4	252	WIITAAHCV	43.992	38
15	5	356	VLNHAAVPL	36.316	39
	6	176	LLPDDKVTA	34.627	40
	7	13	FSFRSLFGL	31.661	41
	8	151	YVSSDNLRV	27.995	42
	9	436	RVTSFLDWI	21.502	43
20	10	234	SLQFQGYHL	21.362	44
	11	181	KVTALHHSV	21.300	45
	12	183	TALHHSVYV	19.658	46
	13	411	RLWKLVGAT	18.494	47
	14	60	LILALAIGL	18.476	48
25	15	227	SQWPWQASL	17.977	49
	16	301	RLGNDIALM	11.426	50
	17	307	ALMKLAGPL	10.275	51
	18	262	DLYLPKSWT	9.837	52
	19	416	LVGATSGFI	9.001	53
30	20	54	SLGIIALIL	8.759	54

HLA A0205

	1	218	IVGGNMSLL	47.600	55
	2	60	LILALAIGL	35.700	48
	3	35	AVAAQILSL	28.000	56
5	4	307	ALMKLAGPL	21.000	51
	5	271	IQVGLVSL	19.040	57
	6	397	CQGDGGPL	16.800	58
	7	227	SQWPWQASL	16.800	49
	8	270	TIQVGLVSL	14.000	59
10	9	56	GIIALILAL	14.000	60
	10	110	RVGGQNAVL	14.000	61
	11	181	KVTALHHSV	12.000	45
	12	151	YVSSDNLRV	12.000	42
	13	356	VLNHAAPPL	11.900	39
15	14	144	AQLGFPSYV	9.600	36
	15	13	FSFRSLFGL	7.560	41
	16	54	SLGIIALIL	7.000	54
	17	234	SLQFQGYHL	7.000	44
	18	217	RIVGGNMSL	7.000	62
20	19	411	RLWKLVGAT	6.000	47
	20	252	WIITAAHCV	6.000	38

HLA A1

	1	130	CSDDWKGHY	37.500	63
	2	8	AVEAPFSFR	9.000	64
25	3	328	NSEENFPDG	2.700	65
	4	3	ENDPPAVEA	2.500	66
	5	98	DCKDGEDEY	2.500	67
	6	346	ATEDGGDAS	2.250	68
	7	360	AAVPLISNK	2.000	69

	8	153	SSDNLRVSS	1.500	70
	9	182	VTALHHSVY	1.250	71
	10	143	CAQLGFPSY	1.000	72
	11	259	CVYDLYLPK	1.000	73
5	12	369	ICNHRDVYG	1.000	74
	13	278	LLDNPAPOH	1.000	75
	14	426	CAEVNKPQV	1.000	76
	15	32	DADAVAAQI	1.000	77
	16	406	VCQERRLWK	1.000	78
10	17	329	SEENFPDGK	0.900	79
	18	303	GNDIALMKL	0.625	80
	19	127	KTMCSDDWK	0.500	81
	20	440	FLDWIHEQM	0.500	82
HLA A24					
15	1	433	VYTRVTSFL	280.000	83
	2	263	LYLPKSWTI	90.000	84
	3	169	EFVSIHLL	42.000	85
	4	217	RIVGGNMSL	12.000	62
	5	296	KYKPKRLGN	12.000	86
20	6	16	RSLFGLDDL	12.000	87
	7	267	KSWTIQVGL	11.200	88
	8	81	RSSFKEIEL	8.800	89
	9	375	VYGGIISPS	8.000	90
	10	110	RVGGQNAVH	8.000	91
25	11	189	VYVREGCAS	7.500	92
	12	60	LILALAIGL	7.200	48
	13	165	QFREEFVSI	7.200	93
	14	271	IQVGLVSLH	7.200	57
	15	56	GIIALILAL	7.200	60

	16	10	EAPFSFRSL	7.200	94
	17	307	ALMKLAGPL	7.200	51
	18	407	CQERRLWKL	6.600	95
	19	356	VLNHAAPVL	6.000	39
5	20	381	SPSMLCAGY	6.000	96
HLA B7					
	1	375	VYGGIISPS	200.000	97
	2	381	SPSMLCAGY	80.000	98
	3	362	VPLISNKIC	80.000	99
10	4	35	AVAAQILSL	60.000	56
	5	373	RDVYGGIIS	40.000	100
	6	307	ALMKLAGPL	36.000	51
	7	283	APSHLVEKI	24.000	101
	8	177	LPDDKV TAL	24.000	102
15	9	47	EVFSQSSSL	20.000	103
	10	110	RVGGQNAVL	20.000	91
	11	218	IVGGNMSLL	20.000	55
	12	36	VAAQILSLL	12.000	104
	13	255	TAAHCVYDL	12.000	105
20	14	10	EAPFSFRSL	12.000	94
	15	138	YANVACAQL	12.000	106
	16	195	CASGHVVTL	12.000	107
	17	215	SSRIVGGNM	10.00	108
	18	298	KPKRLGNDI	8.000	109
25	19	313	GPLTFNEMI	8.000	110
	20	108	CVRVGGQNA	5.000	111
HLA B8					
	1	294	HSKYKPKRL	80.000	112
	2	373	RDVYGGIIS	16.000	100

	3	177	LPDDKVTAL	4.800	102
	4	265	LPKSWTIQV	2.400	113
	5	88	ELITRCDGV	2.400	114
	6	298	KPKRLGNDI	2.000	109
5	7	81	RSSFVKCIEL	2.000	89
	8	375	VYGGIISPS	2.000	97
	9	79	RCRSSFKCI	2.000	115
	10	10	EAPFSFRSL	1.600	94
	11	215	SSRIVGGNM	1.000	108
10	12	36	VAAQILSLL	0.800	104
	13	255	TAAHCVDYDL	0.800	116
	14	381	SPSMLCAGY	0.800	98
	15	195	CASGHVVTI	0.800	107
	16	362	VPLISNKIC	0.800	99
15	17	138	YANVACAQL	0.800	106
	18	207	ACGHRRGYS	0.400	117
	19	154	SDNLRVSSL	0.400	118
	20	47	EVFSQSSSL	0.400	103
HLA B2702					
20	1	300	KRLGNDIAL	180.000	119
	2	435	TRVTSFLDW	100.000	120
	3	376	YGGIISPSM	100.000	121
	4	410	RRLWKLVGA	60.000	122
	5	210	HRRGYSSRI	60.000	123
25	6	227	SQWPWQASL	30.000	49
	7	109	VRVGGQNAV	20.000	124
	8	191	VREGCASGH	20.000	125
	9	78	YRCRSSFKC	20.000	126
	10	113	GQNAVQLQVF	20.000	127

	11	91	TRCDGVSDC	20.000	128
	12	38	AQILSLLPF	20.000	129
	13	211	RRGYSSRIV	18.000	130
	14	216	SRIVGGNMS	10.000	131
5	15	118	LQVFTAASW	10.000	132
	16	370	CNHRDVYGG	10.000	133
	17	393	GVDSCQGDS	10.000	134
	18	235	LQFQGYHLC	10.000	135
	19	271	IQVGLVSL	6.000	57
10	20	408	CQERRLWKL	6.000	95
HLA B4403					
	1	427	AEVNKPGVY	90.000	136
	2	162	LEGQFREEF	40.000	137
	3	9	VEAPFSFRS	24.000	138
15	4	318	NEMIQPVCL	12.000	139
	5	256	AAHCVYDLY	9.000	140
	6	98	DCKDGEDEY	9.000	67
	7	46	FEVFSQSSS	8.000	141
	8	38	AQILSLLPF	7.500	129
20	9	64	LAIGLGIHF	7.500	142
	10	192	REGCASGHV	6.000	143
	11	330	EENFPDGKV	6.000	144
	12	182	VTALHHSVY	6.000	145
	13	408	QERRLWKL	6.000	146
25	14	206	TACGHRRGY	4.500	147
	15	5	DPPAVEAPF	4.500	148
	16	261	YDLYLPKSW	4.500	149
	17	33	ADAVAAQIL	4.500	150
	18	168	EEFVSIDHL	4.000	151

19	304	NDIALMKLA	3.750	152
20	104	DEYRCVRVG	3.600	153

5 Conclusion

In this study, a serine protease was identified by means of a PCR based strategy. By Northern blot, the largest transcript for this gene is approximately 2.4 kb, and it is found to be expressed at high levels in ovarian tumors while found at minimal levels in all other tissues examined. The full-length cDNA encoding a novel multi-domain, cell-surface serine protease was cloned, named TADG-12. The 454 amino acid protein contains a cytoplasmic domain, a type II transmembrane domain, an LDLR-A domain, an SRCR domain and a serine protease domain. Using a semi-quantitative PCR analysis, it was shown that TADG-12 is overexpressed in a majority of tumors studied. Immunohistochemical staining corroborates that in some cases this protein is localized to the cell-surface of tumor cells and this suggests that TADG-12 has some extracellular proteolytic functions. Interestingly, TADG-12 also has a variant splicing form that is present in 35% of the tumors studied. This variant mRNA would lead to a truncated protein that may provide a unique peptide sequence on the surface of tumor cells.

This protein contains two extracellular domains which might confer unusual properties to this multidomain molecule. Although the precise role of LDLR-A function with regard to proteases remains unclear, this domain certainly has the capacity to bind calcium and other positively charged ligands [21,22]. This may play an important role in the regulation of the protease or

subsequent internalization of the molecule. The SRCR domain was originally identified within the macrophage scavenger receptor and functionally described to bind lipoproteins. Not only are SRCR domains capable of binding lipoproteins, but they may also bind to
5 molecules as diverse as polynucleotides [23]. More recent studies have identified members of this domain family in proteins with functions that vary from proteases to cell adhesion molecules involved in maturation of the immune system [24]. In addition, TADG-12, like TMPRSS2 has only four of six cysteine residues
10 conserved within its SRCR domain. This difference may allow for different structural features of these domains that confer unusual ligand binding properties. At this time, only the function of the CD6 encoded SRCR is well documented. In the case of CD6, the SRCR domain binds to the cell adhesion molecule ALCAM [23].
15 This mediation of cell adhesion is a useful starting point for future research on newly identified SRCR domains, however, the possibility of multiple functions for this domain can not be overlooked. SRCR domains are certainly capable of cell adhesion type interactions, but their capacity to bind other types of ligands
20 should be considered.

At this time, the precise role of TADG-12 remains unclear. Substrates have not been identified for the protease domain, nor have ligands been identified for the extracellular LDLR-A and SRCR domains. Figure 8 presents a working model of
25 TADG-12 with the information disclosed in the present invention. Two transcripts are produced which lead to the production of either TADG-12 or the truncated TADG-12V proteins. Either of these proteins is potentially targeted to the cell surface. TADG-12 is capable of becoming an activated serine protease while TADG-

12V is a truncated protein product that if at the cell surface may represent a tumor specific epitope.

The problem with treatment of ovarian cancer today remains the inability to diagnose the disease at an early stage.

5 Identifying genes that are expressed early in the disease process such as proteases that are essential for tumor cell growth [26] is an important step toward improving treatment. With this knowledge, it may be possible to design assays to detect the highly expressed genes such as the TADG-12 protease described

10 here or previously described proteases to diagnose these cancers at an earlier stage. Panels of markers may also provide prognostic information and could lead to therapeutic strategies for individual patients. Alternatively, inhibition of enzymes such as proteases may be an effective means for slowing progression of ovarian

15 cancer and improving the quality of patient life. Other features of TADG-12 and TADG-12V must be considered important to future research too. The extracellular ligand binding domains are natural targets for drug delivery systems. The aberrant peptide associated with the TADG-12V protein may provide an excellent

20 target drug delivery or for immune stimulation.

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Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each
5 individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those
10 inherent therein. The present examples along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will
15 occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A DNA fragment encoding Tumor Associated Differentially-Expressed Gene-12 (TADG-12) protein selected from
5 the group consisting of:

(a) an isolated DNA fragment which encodes a TADG-12 protein;

(b) an isolated DNA fragment which hybridizes to isolated DNA fragment of (a) above and which encodes a TADG-12
10 protein; and

(c) an isolated DNA fragment differing from the isolated DNA fragments of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein.
15

2. The DNA fragment of claim 1, wherein said DNA fragment has the sequence selected from the group consisting of SEQ ID No. 1 and SEQ ID No. 3.

20 3. The DNA fragment of claim 1, wherein said TADG-12 protein has the amino acid sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

4. A vector comprising the DNA fragment of claim 1
25 and regulatory elements necessary for expression of the DNA in a cell.

5. The vector of claim 4, wherein said DNA fragment encodes a TADG-12 protein having the amino acid

sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

6. A host cell transfected with the vector of claim 4,
5 said vector expressing a TADG-12 protein.

7. The host cell of claim 6, wherein said cell is selected from the group consisting of a bacterial cell, a mammalian cell, a plant cell and an insect cell.

10

8. The host cell of claim 7, wherein said bacterial cell is *E. coli*.

9. An antisense oligonucleotide directed against the
15 DNA fragment of claim 1.

10. An isolated and purified TADG-12 protein coded for by DNA selected from the group consisting of:

- (a) isolated DNA which encodes a TADG-12 protein;
20 (b) isolated DNA which hybridizes to isolated DNA of (a) above and which encodes a TADG-12 protein; and
(c) isolated DNA differing from the isolated DNAs of (a) and (b) above in codon sequence due to the degeneracy of the genetic code, and which encodes a TADG-12 protein.

25

11. The isolated and purified TADG-12 protein of claim 10, wherein said TADG-12 protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

12. A method for detecting expression of the TADG-12 protein of claim 10, comprising the steps of:

(a) contacting mRNA obtained from a cell with a labeled hybridization probe; and

5 (b) detecting hybridization of the probe with the mRNA.

13. An antibody directed against the TADG-12 protein of claim 10.

10

14. A method for diagnosing a cancer in an individual, comprising the steps of:

(a) obtaining a biological sample from said individual; and

15 (b) detecting a TADG-12 protein in said sample, wherein the presence of a TADG-12 protein in said sample is indicative of the presence of a cancer in said individual, wherein the absence of a TADG-12 protein in said sample is indicative of the absence of a cancer in said individual.

20

15. The method of claim 14, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

25

16. The method of claim 14, wherein said detection of a TADG-12 protein is by means selected from the group consisting of Northern blot, Western blot, PCR, dot blot, ELIZA

sandwich assay, radioimmunoassay, DNA array chips and flow cytometry.

17. The method of claim 14, wherein said cancer is
5 selected from the group consisting of ovarian cancer, breast cancer, lung cancer, colon cancer, prostate cancer and other cancers in which TADG-12 is overexpressed.

18. A method for detecting malignant hyperplasia in
10 a biological sample, comprising the steps of:

(a) isolating mRNA from said sample; and

(b) detecting TADG-12 mRNA in said sample,
wherein the presence of said TADG-12 mRNA in said sample is
indicative of the presence of malignant hyperplasia, wherein the
15 absence of said TADG-12 mRNA in said sample is indicative of the
absence of malignant hyperplasia.

19. The method of claim 18, further comprising the
step of comparing said TADG-12 mRNA to reference information,
20 wherein said comparison provides a diagnosis of said malignant
hyperplasia.

20. The method of claim 18, further comprising the
step of comparing said TADG-12 mRNA to reference information,
25 wherein said comparison determines a treatment of said
malignant hyperplasia.

21. The method of claim 18, wherein said detection
of TADG-12 mRNA is by PCR amplification.

22. The method of claim 21, wherein said PCR amplification uses primers selected from the group consisting of SEQ ID Nos. 28-31.

5 23. The method of claim 18, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

10 24. A method for detecting malignant hyperplasia in a biological sample, comprising the steps of:

 (a) isolating protein from said sample; and
 (b) detecting a TADG-12 protein in said sample,
 wherein the presence of a TADG-12 protein in said sample is
15 indicative of the presence of malignant hyperplasia, wherein the
 absence of a TADG-12 protein in said sample is indicative of the
 absence of malignant hyperplasia.

 25. The method of claim 24, further comprising the
20 step of comparing said TADG-12 protein to reference information,
 wherein said comparison provides a diagnosis of said malignant
 hyperplasia.

 26. The method of claim 24, further comprising the
25 step of comparing said TADG-12 protein to reference information,
 wherein said comparison determines a treatment of said
 malignant hyperplasia.

27. The method of claim 24, wherein said detection is by immunoaffinity to an antibody, wherein said antibody is directed against a TADG-12 protein.

5 28. The method of claim 24, wherein said biological sample is selected from the group consisting of blood, urine, saliva, tears, interstitial fluid, ascites fluid, tumor tissue biopsy and circulating tumor cells.

10 29. A method of inhibiting expression of endogenous TADG-12 mRNA in a cell, comprising the step of:

 introducing a vector into a cell, wherein said vector comprises a DNA fragment of TADG-12 in opposite orientation operably linked to elements necessary for expression, wherein
15 expression of said vector in said cell produces TADG-12 antisense mRNA, wherein said TADG-12 antisense mRNA hybridizes to endogenous TADG-12 mRNA, thereby inhibiting expression of endogenous TADG-12 mRNA in said cell.

20 30. A method of inhibiting expression of a TADG-12 protein in a cell, comprising the step of:

 introducing an antibody into a cell, wherein said antibody is directed against a TADG-12 protein or fragment thereof, wherein binding of said antibody to said TADG-12 protein
25 or fragment thereof inhibits expression of said TADG-12 protein.

 31. A method of targeted therapy to an individual, comprising the step of:

administering a compound to an individual, wherein said compound has a targeting moiety and a therapeutic moiety, wherein said targeting moiety is specific for a TADG-12 protein.

5 32. The method of claim 31, wherein said targeting moiety is selected from the group consisting of an antibody directed against a TADG-12 protein and a ligand or ligand binding domain that binds a TADG-12 protein.

10 33. The method of claim 32, wherein said TADG-12 protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

15 34. The method of claim 31, wherein said therapeutic moiety is selected from the group consisting of a radioisotope, a toxin, a chemotherapeutic agent, an immune stimulant and a cytotoxic agent.

20 35. The method of claim 31, wherein said individual suffers from a disease selected from the group consisting of ovarian cancer, lung cancer, prostate cancer, colon cancer and other cancers in which TADG-12 is overexpressed.

25 36. A method of vaccinating an individual against TADG-12, comprising the step of inoculating the individual with a TADG-12 protein or fragment thereof, wherein said TADG-12 protein or fragment thereof lacks TADG-12 activity, wherein said inoculation with said TADG-12 protein or fragment thereof elicits

an immune response in said individual, thereby vaccinating said individual against TADG-12.

37. The method of claim 36, wherein said individual
5 has a cancer, is suspected of having a cancer or is at risk of getting a cancer.

38. The method of claim 36, wherein said TADG-12 protein has an amino acid sequence selected from the group consisting of SEQ ID No. 2 and SEQ ID No. 4.

10

39. The method of claim 36, wherein said TADG-12 fragment has a sequence shown in SEQ ID No. 8.

40. The method of claim 36, wherein said TADG-12
15 fragment is a 9-residue fragment selected from the group consisting of SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

41. An immunogenic composition, comprising an
20 immunogenic fragment of a TADG-12 protein and an appropriate adjuvant.

42. The immunogenic composition of claim 41, wherein said immunogenic fragment of a TADG-12 protein has a sequence shown in SEQ ID No. 8.

25

43. The immunogenic composition of claim 41, wherein said immunogenic fragment of a TADG-12 protein is a 9-residue fragment selected from the group consisting of SEQ ID Nos. 35, 36, 55, 56, 83, 84, 97, 98, 119, 120, 122, 123 and 136.

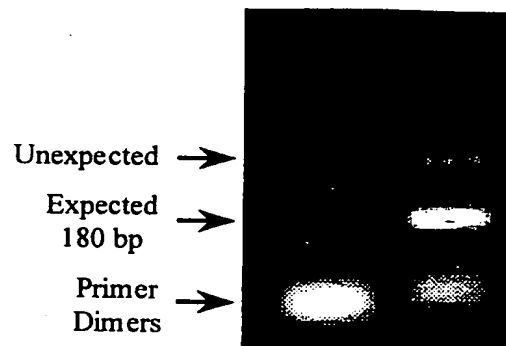


FIG. 1A

TADG12



1 TGGGTGGTGACGGCGGCGCACTGTGTTTATGACTTGTACCTCCCCAAGTCATGGACCATC
W V V T A A **H** C V Y D L Y L P K S W T I

61 CAGGTGGGTCTAGTTTCCCTGTTGGACAATCCAGCCCCATCCCACTTGGTGGAGAAGATT
Q V G L V S L L D N P A P S H L V E K I

121 GTCTACCACAGCAAGTACAAGCCAAAGAGGCTGGGCAACGACATCGCCCTCCTA
V Y H S K Y K P K R L G N **D** I A L L

(SEQ ID NO. 5)
(SEQ ID NO. 6)

TADG12-V



1 GGGTGGTGACGGCGGCGCACTGTGTTTATGAGATTGTAGCTCCTAGAGAAAGGGCAGACA
V V T A A H C V Y E I V A P R E R A D R

61 GAAGAGGAAGGAAGCTCCTGTGCTGGAGGAAACCCACAAAATGAAAGGACCTAGACCTT
R G R K L L C W R K P T K M K G P R P S

121 CCCATAGCTAATTCCAGTGGACCATGTTATGGCAGATACAGGCTTGTACCTCCCCAAGTC
H S * (SEQ ID NO. 8)

181 ATGGACCATCCAGGTGGGTCTAGTTTCCCTGTTGGACAATCCAGCCCCATCCCACTTGGT
241 GGAGAAGATTGTCTACCACAGCAAGTACAAGCCAAAGAGGCTGGGCAACGACATCGCCCT
301 CCTAATCACTAGTGCGGCCGCTGCAGG (SEQ ID NO. 7)

FIG. 1B

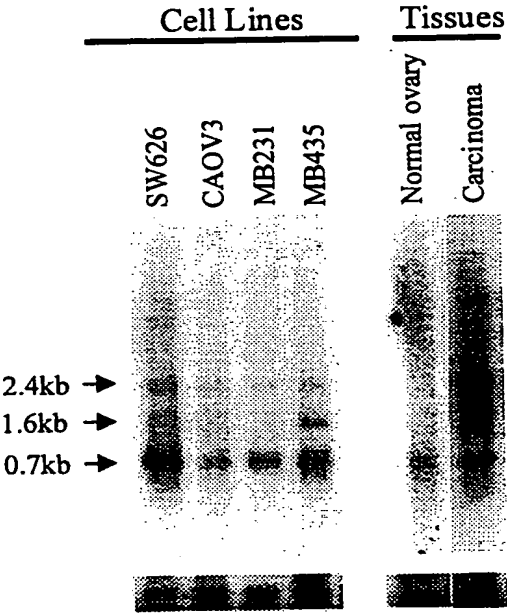


FIG. 2

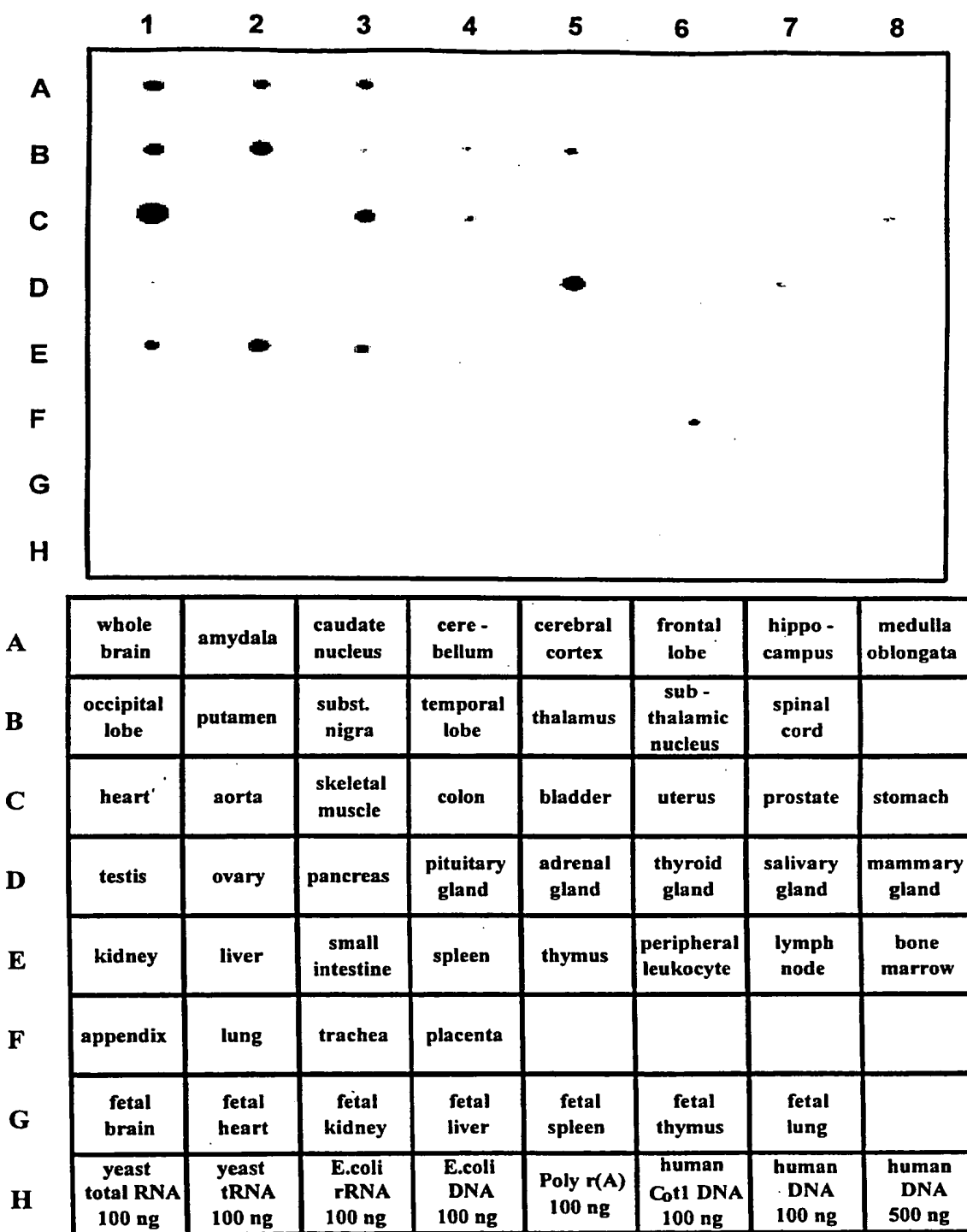


FIG. 3

4/9

Comp8	CEG..FVC	AQTGRCVNRR	LLCNGDND CG	DQSDEAN.C	(SEQ ID NO. 9)
Matr	CPG.QFTC	.RTGRCIRKE	LRCDGWADCT	DHSDELN.C	(SEQ ID NO. 10)
Gp300-1	CQQGYFKC	QSEGQCIPSS	WVCDQDQDCD	DGSDERQDC	(SEQ ID NO. 11)
Gp300-2	CSSHQITC	.SNGQCIPSE	YRCDHVRDCP	DGADE.NDC	(SEQ ID NO. 12)
TADG12	CSGK.YRC	RSSFKEIELI	TRCDGVSDCK	DGEDEYR.C	(SEQ ID NO. 13)
Tmprss2	CSNSGIEC	DSSGTCINPS	NWCDGVSHCP	GGEENR.C	(SEQ ID NO. 14)
Cons	C	C	C	C	DE C

FIG. 5A

BovEntk	VRLVGGSGPH	EGRVEI.FHE	GQWGTVCDDR	WELRGGLVVC	RSLGYKGVQS
MacSR	VRLVGGSGPH	EGRVEI.LHS	GQWGTICDDR	WEVRVGQVVC	RSLGYPGVQA
TADG12	VRVGG...QN	AVLQVFTA..	ASWKTMCSD	WKGHYANVAC	AQLGFP.SYV
Tmprss2	VRLYG...PN	FILQMYSSQR	KSWHPVCQDD	WNENYGRAAC	RDMGYKNNFY
HumEntk	VREFNGTTNN	NGLVRFRIQ.	SIWHTACAEN	WTTQISNDVC	QLLGLGSG..
Cons	VR		W	C	W C
BovEntk	VHKRAYFGKG	TGPIWLNEVF	CFGK..ESSI	EECRIRQWGV	R.ACSHDEDA
MacSR	VHKAHFGQG	TGPIWLNEVF	CFGR..ESSI	EECKIRQWGT	R.ACSHSEDA
TADG12	SSDNLRVSSL	EGQFREEFVS	I.DHLLPDDK	VTALHHSVYV	REGCASGHVV
Tmprss2	SSQGIVDDSG	STSFMKLNTS	A.GNV...DI	YKKLYHS...	.DACSSKAVV
HumEntk	NSSKPIFSTD	GGPFVKLNTA	PDGHLILTPS	QQ.....	...CLQDSL
Cons					C
BovEntk	GVTCT	(SEQ ID NO. 15)			
MacSR	GVTCT	(SEQ ID NO. 16)			
TADG12	TLQCT	(SEQ ID NO. 17)			
Tmprss2	SLRCL	(SEQ ID NO. 18)			
HumEntk	RLQC.	(SEQ ID NO. 19)			
Cons	C				

FIG. 5B


```

Prom  LWVLTAAHCK .....KPNL QVFLGKHNLR QRESSQEQQS VVRAVIHPDY
Try1   QWVVSAGHCY .....KSRI QVRLGEHNIE VLEGNEQFIN AAKIIRHPQY
Kal    QWVLTAAHCF D.GLPLQDVW RIYSGILNLS DITKDTFFSQ IKEIIHQNY
TADG12 LWIITAACHV .YDLXLPKSW TIQVGLV..S LLDNPAPSHL VEKIVYHSKY
Tmprs2 EWIVTAACHV EKPLNNPWHW TAFAGILRQS FMEFYA.GYQ VQKVISHPNY
Heps   DWVLTAACHF PERNRVLSRW RVFAGAVAQA SPHGLQLG.. VQAVVYHGGY
Cons   W   A HC                               G                               H Y

Prom  .....DAAS HDQDIMLLRL ARPAKLSELI QPLPLERDCS ANT..TSCHI
Try1   .....DRKT LNNDIMLIK LSSRAVINARV STISLPTAPP ATG..TKCLI
Kal    .....KVSE GNHDIALIKL QAPLNYTEFQ KPICLPSKGD TSTIYTNCWV
TADG12 .....KPKR LGNDIALMKL AGPLTFNEMI QPVCLPNSEE NFPDGKVCWT
Tmprs2 .....DSKT KNNDIALMKL QKPLTFNDLV KPVCLPNPGM MLQPEQLCWI
Heps   LPFRDPNSEE NSNDIALVHL SSPLPLTEYI QPVCLPAAGQ ALVDGKICTV
Cons   DI L L                               L                               C

Prom  LGWGKTAD.. GDFPDTIQCA YIHLVSREEC EHA..YPGQI TQNMLCAGDE
Try1   SGWGNTASSG ADYPDELQCL DAPVLSQAKC EAS..YPGKI TSNMFCVGFL
Kal    TGWGFSKEK. GEIQNILQKV NIPLVTNEEC QKR.YQDYKI TQRMVCAGYK
TADG12 SGWGAT.EDG GDASPVLNHA AVPLISNKIC NHRDVYGGII SPSMLCAGYL
Tmprs2 SGWGAT.EEK GKTSEVLNAA KVLLIETQRC NSRYVYDNLI TPAMICAGFL
Heps   TGWGNT.QYY GQQAGVLQEA RVPIISNDVC NGADFYGNQI KPKMFCAGYP
Cons   GWG                               C                               I   M C G

Prom  KYGKDSCQGD SGGPLVC (SEQ ID NO. 20)
Try1   EGGKDSCQGD SGGPVVC (SEQ ID NO. 21)
Kal    EGGKDACKGD SGGPLVC (SEQ ID NO. 22)
TADG12 TGGVDSCQGD SGGPLVC (SEQ ID NO. 23)
Tmprs2 QGNVDSCQGD SGGPLVT (SEQ ID NO. 24)
Heps   EGGIDACQGD SGGPFVC (SEQ ID NO. 25)
Cons   D C GD SGGP V

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FIG. 5C

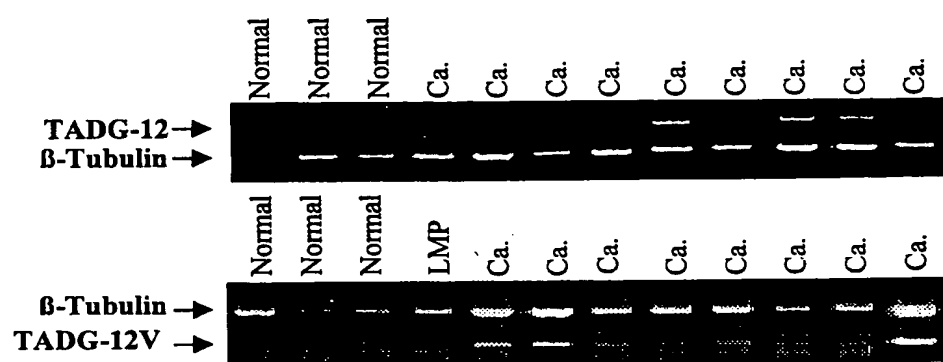


FIG. 6



FIG. 7A



FIG. 7B



FIG. 7C

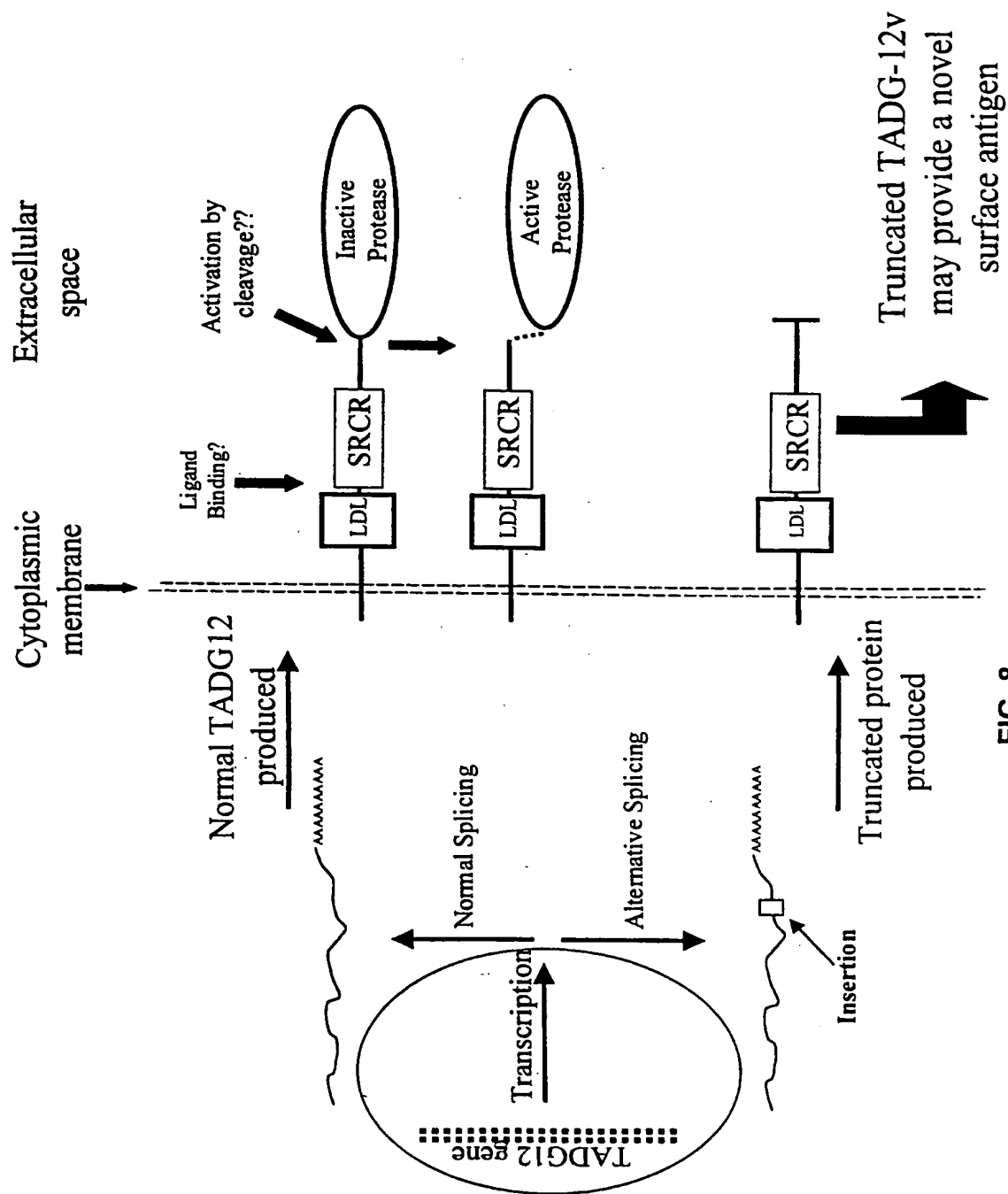


FIG. 8

SEQUENCE LISTING

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      20      25      30
Pro Asp Ala Asp Ala Val Ala Ala Gln Ile Leu Ser Leu Leu Pro
      35      40      45
Phe Glu Val Phe Ser Gln Ser Ser Ser Leu Gly Ile Ile Ala Leu
      50      55      60
Ile Leu Ala Leu Ala Ile Gly Leu Gly Ile His Phe Asp Cys Ser
      65      70      75
Gly Lys Tyr Arg Cys Arg Ser Ser Phe Lys Cys Ile Glu Leu Ile
      80      85      90
Thr Arg Cys Asp Gly Val Ser Asp Cys Lys Asp Gly Glu Asp Glu
      95     100     105
Tyr Arg Cys Val Arg Val Gly Gly Gln Asn Ala Val Leu Gln Val
     110     115     120
Phe Thr Ala Ala Ser Trp Lys Thr Met Cys Ser Asp Asp Trp Lys
     125     130     135
Gly His Tyr Ala Asn Val Ala Cys Ala Gln Leu Gly Phe Pro Ser
     140     145     150
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Phe Arg Glu Glu Phe Val Ser Ile Asp His Leu Leu Pro Asp Asp
     170     175     180
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     185     190     195
Ala Ser Gly His Val Val Thr Leu Gln Cys Thr Ala Cys Gly His
     200     205     210
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His	Leu	Val	Glu	Lys	Ile	Val	Tyr	His	Ser	Lys	Tyr	Lys	Pro	Lys	
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gagttccggc accagtagcg ggcccgaag aggcaccctt ccatctgatt 1800
ccagcacaac cttcaagctg ctttttgttt tttgtttttt tgaggtggag 1850
tctcgctctg ttgcccaggc tggagtgcag tggcgaaata ccctgctcac 1900
tgcagctctc gcttccctgg ttcaagcgat tctcttgctc cagcttcccc 1950
agtactgggg accacaggtg ccgcgcacca caccacaacta atttttgtat 2000
ttttagtaga gacagggttt caccatgttg gccaggctgc tctcaaacc 2050
ctgacctcaa atgatgtgcc tgcttcagcc tcccacagtg ctgggattac 2100
aggcatgggc caccacgcct agcctcacgc tcttttctga tcttactaa 2150
gaacaaaaga agcagcaact tgcaagggcg gcctttccca ctggtccatc 2200
tggttttctc tccagggtct tgcaaaattc ctgacgagat aagcagttat 2250
gtgacctcac gtgcaaagcc accaacagcc actcagaaaa gacgtaccag 2300
cccagaagtg cagaactgca gtcactgcac gttttcatct ttagggacca 2350
gaaccaaacc caccctttct acttccaaga cttattttca catgtgggga 2400
ggttaatcta ggaatgactc gtttaaggcc tattttcatg atttctttgt 2450
agcatttggg gcttgacgta ttattgtcct ttgattccaa ataatatgtt 2500
tccttccctc aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa 2544

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<210>      4
<211>      294
<212>      PRT
<213>      Homo sapiens
<220>
<223>      complete amino acid sequence of TADG-12
variant protein
<400>      4

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Met Gly Glu Asn Asp Pro Pro Ala Val Glu Ala Pro Phe Ser Phe
      5      10
Arg Ser Leu Phe Gly Leu Asp Asp Leu Lys Ile Ser Pro Val Ala
      20      25
Pro Asp Ala Asp Ala Val Ala Ala Gln Ile Leu Ser Leu Leu Pro
      35      40
Phe Glu Val Phe Ser Gln Ser Ser Ser Leu Gly Ile Ile Ala Leu
      50      55
Ile Leu Ala Leu Ala Ile Gly Leu Gly Ile His Phe Asp Cys Ser
      65      70
Gly Lys Tyr Arg Cys Arg Ser Ser Phe Lys Cys Ile Glu Leu Ile

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				80					85				90	
Thr	Arg	Cys	Asp	Gly	Val	Ser	Asp	Cys	Lys	Asp	Gly	Glu	Asp	Glu
				95					100					105
Tyr	Arg	Cys	Val	Arg	Val	Gly	Gly	Gln	Asn	Ala	Val	Leu	Gln	Val
				110					115					120
Phe	Thr	Ala	Ala	Ser	Trp	Lys	Thr	Met	Cys	Ser	Asp	Asp	Trp	Lys
				125					130					135
Gly	His	Tyr	Ala	Asn	Val	Ala	Cys	Ala	Gln	Leu	Gly	Phe	Pro	Ser
				140					145					150
Tyr	Val	Ser	Ser	Asp	Asn	Leu	Arg	Val	Ser	Ser	Leu	Glu	Gly	Gln
				155					160					165
Phe	Arg	Glu	Glu	Phe	Val	Ser	Ile	Asp	His	Leu	Leu	Pro	Asp	Asp
				170					175					180
Lys	Val	Thr	Ala	Leu	His	His	Ser	Val	Tyr	Val	Arg	Glu	Gly	Cys
				185					190					195
Ala	Ser	Gly	His	Val	Val	Thr	Leu	Gln	Cys	Thr	Ala	Cys	Gly	His
				200					205					210
Arg	Arg	Gly	Tyr	Ser	Ser	Arg	Ile	Val	Gly	Gly	Asn	Met	Ser	Leu
				215					220					225
Leu	Ser	Gln	Trp	Pro	Trp	Gln	Ala	Ser	Leu	Gln	Phe	Gln	Gly	Tyr
				230					235					240
His	Leu	Cys	Gly	Gly	Ser	Val	Ile	Thr	Pro	Leu	Trp	Ile	Ile	Thr
				245					250					255
Ala	Ala	His	Cys	Val	Tyr	Glu	Ile	Val	Ala	Pro	Arg	Glu	Arg	Ala
				260					265					270
Asp	Arg	Arg	Gly	Arg	Lys	Leu	Leu	Cys	Trp	Arg	Lys	Pro	Thr	Lys
				275					280					285
Met	Lys	Gly	Pro	Arg	Pro	Ser	His	Ser						
				290										

<210> 5
 <211> 174
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> nucleotide sequence of the subclone containing
 the 180 bp band from the PCR product for TADG-12
 <400> 5

tggtgtggtga	cggcggcgca	ctgtgtttat	gacttgtacc	tccccaagtc	50
atggaccatc	caggtgggtc	tagtttcct	gttggaacat	ccagcccat	100
cccacttggt	ggagaagatt	gtctaccaca	gcaagtacaa	gccaaagagg	150
ctgggcaacg	acatcgccct	ccta			174

<210> 6
 <211> 58
 <212> PRT
 <213> Artificial sequence
 <220>
 <223> deduced amino acid sequence of the 180 bp band
 from the PCR product for TADG-12
 <400> 6

Trp	Val	Val	Thr	Ala	Ala	His	Cys	Val	Tyr	Asp	Leu	Tyr	Leu	Pro
				5					10					15
Lys	Ser	Trp	Thr	Ile	Gln	Val	Gly	Leu	Val	Ser	Leu	Leu	Asp	Asn

				20					25					30
Pro	Ala	Pro	Ser	His	Leu	Val	Glu	Lys	Ile	Val	Tyr	His	Ser	Lys
				35					40					45
Tyr	Lys	Pro	Lys	Arg	Leu	Gly	Asn	Asp	Ile	Ala	Leu	Leu		
				50					55					

<210> 7
 <211> 328
 <212> DNA
 <213> Artificial sequence
 <220>
 <223> nucleotide sequence of the subclone containing the 300 bp band from the PCR product for TADG-12 variant, which contains an additional insert of 133 bases
 <400> 7

gggtggtgac	ggcggcgac	tgtgtttatg	agattgtagc	tcctagagaa	50
agggcagaca	gaagaggaag	gaagctcctg	tgctggagga	aacccacaaa	100
aatgaaagga	cctagacctt	cccatagcta	attccagtgg	accatgttat	150
ggcagataca	ggcttgtagc	tccccaagtc	atggaccatc	caggtgggtc	200
tagtttcct	gttggacaat	ccagcccat	cccacttggt	ggagaagatt	250
gtctaccaca	gcaagtacaa	gccaaagagg	ctgggcaacg	acatcgccct	300
cctaatacct	agtgcggccg	cctgcagg			328

<210> 8
 <211> 42
 <212> PRT
 <213> Artificial sequence
 <220>
 <223> deduced amino acid sequence of the 300 bp band from the PCR product for TADG-12 variant, which is a truncated form of TADG-12
 <400> 8

Val	Val	Thr	Ala	Ala	His	Cys	Val	Tyr	Glu	Ile	Val	Ala	Pro	Arg
				5					10					15
Glu	Arg	Ala	Asp	Arg	Arg	Gly	Arg	Lys	Leu	Leu	Cys	Trp	Arg	Lys
				20					25					30
Pro	Thr	Lys	Met	Lys	Gly	Pro	Arg	Pro	Ser	His	Ser			
				35					40					

<210> 9
 <211> 34
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> LDLR-A domain of the complement subunit C8 (Compc8)
 <400> 9

Cys	Glu	Gly	Phe	Val	Cys	Ala	Gln	Thr	Gly	Arg	Cys	Val	Asn	Arg
				5					10					15
Arg	Leu	Leu	Cys	Asn	Gly	Asp	Asn	Asp	Cys	Gly	Asp	Gln	Ser	Asp
				20					25					30

Glu Ala Asn Cys

<210> 10
 <211> 34
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> LDLR-A domain of the serine protease
 matriptase (Matr)
 <400> 10

Cys Pro Gly Gln Phe Thr Cys Arg Thr Gly Arg Cys Ile Arg Lys
 5 10 15
 Glu Leu Arg Cys Asp Gly Trp Ala Asp Cys Thr Asp His Ser Asp
 20 25 30
 Glu Leu Asn Cys

<210> 11
 <211> 37
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> LDLR-A domain of the glycoprotein GP300
 (Gp300-1)
 <400> 11

Cys Gln Gln Gly Tyr Phe Lys Cys Gln Ser Glu Gly Gln Cys Ile
 5 10 15
 Pro Ser Ser Trp Val Cys Asp Gln Asp Gln Asp Cys Asp Asp Gly
 20 25 30
 Ser Asp Glu Arg Gln Asp Cys
 35

<210> 12
 <211> 35
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> LDLR-A domain of the glycoprotein GP300
 (Gp300-2)
 <400> 12

Cys Ser Ser His Gln Ile Thr Cys Ser Asn Gly Gln Cys Ile Pro
 5 10 15
 Ser Glu Tyr Arg Cys Asp His Val Arg Asp Cys Pro Asp Gly Ala
 20 25 30
 Asp Glu Asn Asp Cys
 35

<210> 13
 <211> 35

SEQ 7/41

<212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <222> 74...108
 <223> LDLR-A domain of TADG-12
 <400> 13

Cys	Ser	Gly	Lys	Tyr	Arg	Cys	Arg	Ser	Ser	Phe	Lys	Cys	Ile	Glu
				5					10					15
Leu	Ile	Thr	Arg	Cys	Asp	Gly	Val	Ser	Asp	Cys	Lys	Asp	Gly	Glu
				20					25					30
Asp	Glu	Tyr	Arg	Cys										
				35										

<210> 14
 <211> 36
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> LDLR-A domain of the serine protease TMPRSS2
 Tmprss2
 <400> 14

Cys	Ser	Asn	Ser	Gly	Ile	Glu	Cys	Asp	Ser	Ser	Gly	Thr	Cys	Ile
				5					10					15
Asn	Pro	Ser	Asn	Trp	Cys	Asp	Gly	Val	Ser	His	Cys	Pro	Gly	Gly
				20					25					30
Glu	Asp	Glu	Asn	Arg	Cys									
				35										

<210> 15
 <211> 101
 <212> PRT
 <213> *Bos taurus*
 <220>
 <221> DOMAIN
 <223> SRCR domain of bovine enterokinase (BovEntk)
 <400> 15

Val	Arg	Leu	Val	Gly	Gly	Ser	Gly	Pro	His	Glu	Gly	Arg	Val	Glu
				5					10					15
Ile	Phe	His	Glu	Gly	Gln	Trp	Gly	Thr	Val	Cys	Asp	Asp	Arg	Trp
				20					25					30
Glu	Leu	Arg	Gly	Gly	Leu	Val	Val	Cys	Arg	Ser	Leu	Gly	Tyr	Lys
				35					40					45
Gly	Val	Gln	Ser	Val	His	Lys	Arg	Ala	Tyr	Phe	Gly	Lys	Gly	Thr
				50					55					60
Gly	Pro	Ile	Trp	Leu	Asn	Glu	Val	Phe	Cys	Phe	Gly	Lys	Glu	Ser
				65					70					75
Ser	Ile	Glu	Glu	Cys	Arg	Ile	Arg	Gln	Trp	Gly	Val	Arg	Ala	Cys
				80					85					90
Ser	His	Asp	Glu	Asp	Ala	Gly	Val	Thr	Cys	Thr				
				95					100					

<210> 16
 <211> 101
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> SRCR domain of human macrophage scavenger
 receptor (MacSR)
 <400> 16

Val	Arg	Leu	Val	Gly	Gly	Ser	Gly	Pro	His	Glu	Gly	Arg	Val	Glu
				5					10					15
Ile	Leu	His	Ser	Gly	Gln	Trp	Gly	Thr	Ile	Cys	Asp	Asp	Arg	Trp
				20					25					30
Glu	Val	Arg	Val	Gly	Gln	Val	Val	Cys	Arg	Ser	Leu	Gly	Tyr	Pro
				35					40					45
Gly	Val	Gln	Ala	Val	His	Lys	Ala	Ala	His	Phe	Gly	Gln	Gly	Thr
				50					55					60
Gly	Pro	Ile	Trp	Leu	Asn	Glu	Val	Phe	Cys	Phe	Gly	Arg	Glu	Ser
				65					70					75
Ser	Ile	Glu	Glu	Cys	Lys	Ile	Arg	Gln	Trp	Gly	Thr	Arg	Ala	Cys
				80					85					90
Ser	His	Ser	Glu	Asp	Ala	Gly	Val	Thr	Cys	Thr				
				95					100					

<210> 17
 <211> 98
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <222> 109...206
 <223> SRCR domain of TADG-12 (TADG12)
 <400> 17

Val	Arg	Val	Gly	Gly	Gln	Asn	Ala	Val	Leu	Gln	Val	Phe	Thr	Ala
				5					10					15
Ala	Ser	Trp	Lys	Thr	Met	Cys	Ser	Asp	Asp	Trp	Lys	Gly	His	Tyr
				20					25					30
Ala	Asn	Val	Ala	Cys	Ala	Gln	Leu	Gly	Phe	Pro	Ser	Tyr	Val	Ser
				35					40					45
Ser	Asp	Asn	Leu	Arg	Val	Ser	Ser	Leu	Glu	Gly	Gln	Phe	Arg	Glu
				50					55					60
Glu	Phe	Val	Ser	Ile	Asp	His	Leu	Leu	Pro	Asp	Asp	Lys	Val	Thr
				65					70					75
Ala	Leu	His	His	Ser	Val	Tyr	Val	Arg	Glu	Gly	Cys	Ala	Ser	Gly
				80					85					90
His	Val	Val	Thr	Leu	Gln	Cys	Thr							
				95										

<210> 18
 <211> 94
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN

<223> SRCR domain of the serine protease TMPRSS2
(Tmprss2)
<400> 18

Val	Arg	Leu	Tyr	Gly	Pro	Asn	Phe	Ile	Leu	Gln	Met	Tyr	Ser	Ser
				5					10					15
Gln	Arg	Lys	Ser	Trp	His	Pro	Val	Cys	Gln	Asp	Asp	Trp	Asn	Glu
				20					25					30
Asn	Tyr	Gly	Arg	Ala	Ala	Cys	Arg	Asp	Met	Gly	Tyr	Lys	Asn	Asn
				35					40					45
Phe	Tyr	Ser	Ser	Gln	Gly	Ile	Val	Asp	Asp	Ser	Gly	Ser	Thr	Ser
				50					55					60
Phe	Met	Lys	Leu	Asn	Thr	Ser	Ala	Gly	Asn	Val	Asp	Ile	Tyr	Lys
				65					70					75
Lys	Leu	Tyr	His	Ser	Asp	Ala	Cys	Ser	Ser	Lys	Ala	Val	Val	Ser
				80					85					90
Leu	Arg	Cys	Leu											

<210> 19
<211> 90
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> SRCR domain of human enterokinase (HumEntk)
<400> 19

Val	Arg	Phe	Phe	Asn	Gly	Thr	Thr	Asn	Asn	Asn	Gly	Leu	Val	Arg
				5					10					15
Phe	Arg	Ile	Gln	Ser	Ile	Trp	His	Thr	Ala	Cys	Ala	Glu	Asn	Trp
				20					25					30
Thr	Thr	Gln	Ile	Ser	Asn	Asp	Val	Cys	Gln	Leu	Leu	Gly	Leu	Gly
				35					40					45
Ser	Gly	Asn	Ser	Ser	Lys	Pro	Ile	Phe	Ser	Thr	Asp	Gly	Gly	Pro
				50					55					60
Phe	Val	Lys	Leu	Asn	Thr	Ala	Pro	Asp	Gly	His	Leu	Ile	Leu	Thr
				65					70					75
Pro	Ser	Gln	Gln	Cys	Leu	Gln	Asp	Ser	Leu	Ile	Arg	Leu	Gln	Cys
				80					85					90

<210> 20
<211> 149
<212> PRT
<213> *Homo sapiens*
<220>
<221> DOMAIN
<223> protease domain of protease M (ProM)
<400> 20

Leu	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Lys	Lys	Pro	Asn	Leu	Gln
				5					10					15
Val	Phe	Leu	Gly	Lys	His	Asn	Leu	Arg	Gln	Arg	Glu	Ser	Ser	Gln
				20					25					30
Glu	Gln	Ser	Ser	Val	Val	Arg	Ala	Val	Ile	His	Pro	Asp	Tyr	Asp
				35					40					45
Ala	Ala	Ser	His	Asp	Gln	Asp	Ile	Met	Leu	Leu	Arg	Leu	Ala	Arg

Pro	Ala	Lys	Leu	50	Ser	Glu	Leu	Ile	Gln	55	Pro	Leu	Pro	Leu	Glu	60	Arg
Asp	Cys	Ser	Ala	65	Asn	Thr	Thr	Ser	Cys	70	His	Ile	Leu	Gly	Trp	75	Gly
Lys	Thr	Ala	Asp	80	Gly	Asp	Phe	Pro	Asp	85	Thr	Ile	Gln	Cys	Ala	90	Tyr
Ile	His	Leu	Val	95	Ser	Arg	Glu	Glu	Cys	100	Glu	His	Ala	Tyr	Pro	105	Gly
Gln	Ile	Thr	Gln	110	Asn	Met	Leu	Cys	Ala	115	Gly	Asp	Glu	Lys	Tyr	120	Gly
Lys	Asp	Ser	Cys	125	Gln	Gly	Asp	Ser	Gly	130	Gly	Pro	Leu	Val	Cys	135	
				140						145							

<210> 21
 <211> 151
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> protease domain of trypsinogen I (Try1)
 <400> 21

Gln	Trp	Val	Val	5	Ser	Ala	Gly	His	Cys	10	Tyr	Lys	Ser	Arg	Ile	Gln	15
Val	Arg	Leu	Gly	20	Glu	His	Asn	Ile	Glu	25	Val	Leu	Glu	Gly	Asn	Glu	30
Gln	Phe	Ile	Asn	35	Ala	Ala	Lys	Ile	Ile	40	Arg	His	Pro	Gln	Tyr	Asp	45
Arg	Lys	Thr	Leu	50	Asn	Asn	Asp	Ile	Met	55	Leu	Ile	Lys	Leu	Ser	Ser	60
Arg	Ala	Val	Ile	65	Asn	Ala	Arg	Val	Ser	70	Thr	Ile	Ser	Leu	Pro	Thr	75
Ala	Pro	Pro	Ala	80	Thr	Gly	Thr	Lys	Cys	85	Leu	Ile	Ser	Gly	Trp	Gly	90
Asn	Thr	Ala	Ser	95	Ser	Gly	Ala	Asp	Tyr	100	Pro	Asp	Glu	Leu	Gln	Cys	105
Leu	Asp	Ala	Pro	110	Val	Leu	Ser	Gln	Ala	115	Lys	Cys	Glu	Ala	Ser	Tyr	120
Pro	Gly	Lys	Ile	125	Thr	Ser	Asn	Met	Phe	130	Cys	Val	Gly	Phe	Leu	Glu	135
Gly	Gly	Lys	Asp	140	Ser	Cys	Gln	Gly	Asp	145	Ser	Gly	Gly	Pro	Val	Val	150
Cys																	

<210> 22
 <211> 158
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> protease domain of plasma kallikrein (Kal)
 <400> 22

Gln Trp Val Leu Thr Ala Ala His Cys Phe Asp Gly Leu Pro Leu

	5		10		15
Gln Asp Val Trp	Arg Ile Tyr Ser Gly	Ile Leu Asn Leu Ser	Asp		
	20		25		30
Ile Thr Lys Asp	Thr Pro Phe Ser Gln	Ile Lys Glu Ile Ile	Ile		
	35		40		45
His Gln Asn Tyr	Lys Val Ser Glu Gly	Asn His Asp Ile Ala	Leu		
	50		55		60
Ile Lys Leu Gln	Ala Pro Leu Asn Tyr	Thr Glu Phe Gln Lys	Pro		
	65		70		75
Ile Cys Leu Pro	Ser Lys Gly Asp Thr	Ser Thr Ile Tyr Thr	Asn		
	80		85		90
Cys Trp Val Thr	Gly Trp Gly Phe Ser	Lys Glu Lys Gly Glu	Ile		
	95		100		105
Gln Asn Ile Leu	Gln Lys Val Asn Ile	Pro Leu Val Thr Asn	Glu		
	110		115		120
Glu Cys Gln Lys	Arg Tyr Gln Asp Tyr	Lys Ile Thr Gln Arg	Met		
	125		130		135
Val Cys Ala Gly	Tyr Lys Glu Gly Gly	Lys Asp Ala Cys Lys	Gly		
	140		145		150
Asp Ser Gly Gly	Pro Leu Val Cys				
	155				

<210> 23
 <211> 157
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> protease domain of TADG-12 (TADG12)
 <400> 23

Leu Trp Ile Ile	Thr Ala Ala His Cys	Val Tyr Asp Leu Tyr	Leu		
	5		10		15
Pro Lys Ser Trp	Thr Ile Gln Val Gly	Leu Val Ser Leu Leu	Asp		
	20		25		30
Asn Pro Ala Pro	Ser His Leu Val Glu	Lys Ile Val Tyr His	Ser		
	35		40		45
Lys Tyr Lys Pro	Lys Arg Leu Gly Asn	Asp Ile Ala Leu Met	Lys		
	50		55		60
Leu Ala Gly Pro	Leu Thr Phe Asn Glu	Met Ile Gln Pro Val	Cys		
	65		70		75
Leu Pro Asn Ser	Glu Glu Asn Phe Pro	Asp Gly Lys Val Cys	Trp		
	80		85		90
Thr Ser Gly Trp	Gly Ala Thr Glu Asp	Gly Gly Asp Ala Ser	Pro		
	95		100		105
Val Leu Asn His	Ala Ala Val Pro Leu	Ile Ser Asn Lys Ile	Cys		
	110		115		120
Asn His Arg Asp	Val Tyr Gly Gly Ile	Ile Ser Pro Ser Met	Leu		
	125		130		135
Cys Ala Gly Tyr	Leu Thr Gly Gly Val	Asp Ser Cys Gln Gly	Asp		
	140		145		150
Ser Gly Gly Pro	Leu Val Cys				
	155				

<210> 24
 <211> 159

<212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> protease domain of TMPRSS2 (Tmprss2)
 <400> 24

Glu	Trp	Ile	Val	Thr	Ala	Ala	His	Cys	Val	Glu	Lys	Pro	Leu	Asn	
				5					10					15	
Asn	Pro	Trp	His	Trp	Thr	Ala	Phe	Ala	Gly	Ile	Leu	Arg	Gln	Ser	
				20					25					30	
Phe	Met	Phe	Tyr	Gly	Ala	Gly	Tyr	Gln	Val	Gln	Lys	Val	Ile	Ser	
				35					40					45	
His	Pro	Asn	Tyr	Asp	Ser	Lys	Thr	Lys	Asn	Asn	Asp	Ile	Ala	Leu	
				50					55					60	
Met	Lys	Leu	Gln	Lys	Pro	Leu	Thr	Phe	Asn	Asp	Leu	Val	Lys	Pro	
				65					70					75	
Val	Cys	Leu	Pro	Asn	Pro	Gly	Met	Met	Leu	Gln	Pro	Glu	Gln	Leu	
				80					85					90	
Cys	Trp	Ile	Ser	Gly	Trp	Gly	Ala	Thr	Glu	Glu	Lys	Gly	Lys	Thr	
				95					100					105	
Ser	Glu	Val	Leu	Asn	Ala	Ala	Lys	Val	Leu	Leu	Ile	Glu	Thr	Gln	
				110					115					120	
Arg	Cys	Asn	Ser	Arg	Tyr	Val	Tyr	Asp	Asn	Leu	Ile	Thr	Pro	Ala	
				125					130					135	
Met	Ile	Cys	Ala	Gly	Phe	Leu	Gln	Gly	Asn	Val	Asp	Ser	Cys	Gln	
				140					145					150	
Gly	Asp	Ser	Gly	Gly	Pro	Leu	Val	Thr							
				155											

<210> 25
 <211> 164
 <212> PRT
 <213> *Homo sapiens*
 <220>
 <221> DOMAIN
 <223> protease domain of Hepsin (Heps)
 <400> 25

Asp	Trp	Val	Leu	Thr	Ala	Ala	His	Cys	Phe	Pro	Glu	Arg	Asn	Arg	
				5					10					15	
Val	Leu	Ser	Arg	Trp	Arg	Val	Phe	Ala	Gly	Ala	Val	Ala	Gln	Ala	
				20					25					30	
Ser	Pro	His	Gly	Leu	Gln	Leu	Gly	Val	Gln	Ala	Val	Val	Tyr	His	
				35					40					45	
Gly	Gly	Tyr	Leu	Pro	Phe	Arg	Asp	Pro	Asn	Ser	Glu	Glu	Asn	Ser	
				50					55					60	
Asn	Asp	Ile	Ala	Leu	Val	His	Leu	Ser	Ser	Pro	Leu	Pro	Leu	Thr	
				65					70					75	
Glu	Tyr	Ile	Gln	Pro	Val	Cys	Leu	Pro	Ala	Ala	Gly	Gln	Ala	Leu	
				80					85					90	
Val	Asp	Gly	Lys	Ile	Cys	Thr	Val	Thr	Gly	Trp	Gly	Asn	Thr	Gln	
				95					100					105	
Tyr	Tyr	Gly	Gln	Gln	Ala	Gly	Val	Leu	Gln	Glu	Ala	Arg	Val	Pro	
				110					115					120	
Ile	Ile	Ser	Asn	Asp	Val	Cys	Asn	Gly	Ala	Asp	Phe	Tyr	Gly	Asn	

	125		130		135
Gln Ile Lys Pro	Lys Met Phe Cys Ala	Gly Tyr Pro Glu Gly	Gly		
	140		145		150
Ile Asp Ala Cys	Gln Gly Asp Ser Gly	Gly Pro Phe Val Cys			
	155		160		

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 <222> 6, 9, 12, 15, 18
 <223> forward redundant primer for the consensus
 sequences of amino acids surrounding the catalytic
 triad for serine proteases, n = inosine
 <400> 26

tgggtngtna cngcngcnca ytg 23

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 sequences of amino acids surrounding the catalytic
 triad for serine proteases, n = inosine
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arnarngcna tntcnttncc 20

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 used for quantitative PCR
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gaaacatgtc cttgctctcg 20

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 used for quantitative PCR
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actaacttcc acagcctcct

20

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 variant (TADG-12V) used for quantitative PCR
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tccaggtggg tctagtttcc

20

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 variant (TADG-12V) used for quantitative PCR
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20

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 <223> forward oligonucleotide primer for β -tubulin
 used as an internal control for quantitative PCR
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cgcatcaacg tgtactacaa

20

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 used as an internal control for quantitative PCR
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tacgagctgg tggactgaga

20

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 <223> a poly-lysine linked multiple antigen peptide

derived from the TADG-12 carboxy-terminal protein sequence, present in full length TADG-12, but not in TADG-12V

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Trp Ile His Glu Gln Met Glu Arg Asp Leu Lys Thr
5 10

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Ile Leu Ser Leu Leu Pro Phe Glu Val
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Ala Gln Leu Gly Phe Pro Ser Tyr Val
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Leu Leu Ser Gln Trp Pro Trp Gln Ala
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Trp Ile Ile Thr Ala Ala His Cys Val
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Val Leu Asn His Ala Ala Val Pro Leu
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Leu Leu Pro Asp Asp Lys Val Thr Ala
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Phe Ser Phe Arg Ser Leu Phe Gly Leu
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Tyr Val Ser Ser Asp Asn Leu Arg Val
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Arg Val Thr Ser Phe Leu Asp Trp Ile
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Ser Leu Gln Phe Gln Gly Tyr His Leu
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Lys Val Thr Ala Leu His His Ser Val
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Thr Ala Leu His His Ser Val Tyr Val
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Arg Leu Trp Lys Leu Val Gly Ala Thr
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Leu Ile Leu Ala Leu Ala Ile Gly Leu
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Ser Gln Trp Pro Trp Gln Ala Ser Leu
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Arg Leu Gly Asn Asp Ile Ala Leu Met
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Ala Leu Met Lys Leu Ala Gly Pro Leu
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Asp Leu Tyr Leu Pro Lys Ser Trp Thr
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Leu Val Gly Ala Thr Ser Phe Gly Ile
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Ser Leu Gly Ile Ile Ala Leu Ile Leu
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Ile Val Gly Gly Asn Met Ser Leu Leu
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Ala Val Ala Ala Gln Ile Leu Ser Leu
5

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Ile Gln Val Gly Leu Val Ser Leu Leu
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Cys Gln Gly Asp Ser Gly Gly Pro Leu
5

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Thr Ile Gln Val Gly Leu Val Ser Leu
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Gly Ile Ile Ala Leu Ile Leu Ala Leu
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Arg Val Gly Gly Gln Asn Ala Val Leu
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Arg Ile Val Gly Gly Asn Met Ser Leu
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Cys Ser Asp Asp Trp Lys Gly His Tyr
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Ala Val Glu Ala Pro Phe Ser Phe Arg
5

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Asn Ser Glu Glu Asn Phe Pro Asp Gly
5

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Glu Asn Asp Pro Pro Ala Val Glu Ala
5

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Asp Cys Lys Asp Gly Glu Asp Glu Tyr
5

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Ala Thr Glu Asp Gly Gly Asp Ala Ser
5

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Ala Ala Val Pro Leu Ile Ser Asn Lys
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Ser Ser Asp Asn Leu Arg Val Ser Ser
5

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Val Thr Ala Leu His His Ser Val Tyr
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Cys Ala Gln Leu Gly Phe Pro Ser Tyr
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Cys Val Tyr Asp Leu Tyr Leu Pro Lys
5

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Ile Cys Asn His Arg Asp Val Tyr Gly
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Leu Leu Asp Asn Pro Ala Pro Ser His
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Cys Ala Glu Val Asn Lys Pro Gly Val
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Asp Ala Asp Ala Val Ala Ala Gln Ile
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Val Cys Gln Glu Arg Arg Leu Trp Lys
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Ser Glu Glu Asn Phe Pro Asp Gly Lys
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Gly Asn Asp Ile Ala Leu Met Lys Leu
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Lys Thr Met Cys Ser Asp Asp Trp Lys
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Phe Leu Asp Trp Ile His Glu Gln Met
5

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Val Tyr Thr Arg Val Thr Ser Phe Leu
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Leu Tyr Leu Pro Lys Ser Trp Thr Ile
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Glu Phe Val Ser Ile Asp His Leu Leu
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Lys Tyr Lys Pro Lys Arg Leu Gly Asn
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Arg Ser Leu Phe Gly Leu Asp Asp Leu
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Lys Ser Trp Thr Ile Gln Val Gly Leu
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Arg Ser Ser Phe Lys Cys Ile Glu Leu
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Val Tyr Gly Gly Ile Ile Ser Pro Ser
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Arg Val Gly Gly Gln Asn Ala Val Leu
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Val Tyr Val Arg Glu Gly Cys Ala Ser
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Gln Phe Arg Glu Glu Phe Val Ser Ile
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Glu Ala Pro Phe Ser Phe Arg Ser Leu
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Cys Gln Glu Arg Arg Leu Trp Lys Leu
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Ser Pro Ser Met Leu Cys Ala Gly Tyr
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Val Tyr Gly Gly Ile Ile Ser Pro Ser
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Ser Pro Ser Met Leu Cys Ala Gly Tyr
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Val Pro Leu Ile Ser Asn Lys Ile Cys
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Arg Asp Val Tyr Gly Gly Ile Ile Ser
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Ala Pro Ser His Leu Val Glu Lys Ile
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Leu Pro Asp Asp Lys Val Thr Ala Leu
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Glu Val Phe Ser Gln Ser Ser Ser Leu
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SEQ 30/41

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Val Ala Ala Gln Ile Leu Ser Leu Leu
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Thr Ala Ala His Cys Val Tyr Asp Leu
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Tyr Ala Asn Val Ala Cys Ala Gln Leu
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Cys Ala Ser Gly His Val Val Thr Leu
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Ser Ser Arg Ile Val Gly Gly Asn Met
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Lys Pro Lys Arg Leu Gly Asn Asp Ile
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Gly Pro Leu Thr Phe Asn Glu Met Ile
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Cys Val Arg Val Gly Gly Gln Asn Ala
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His Ser Lys Tyr Lys Pro Lys Arg Leu
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Leu Pro Lys Ser Trp Thr Ile Gln Val
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<222> 88...96

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Glu Leu Ile Thr Arg Cys Asp Gly Val
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<222> 79...87

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Arg Cys Arg Ser Ser Phe Lys Cys Ile
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<222> 255...263

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Thr Ala Ala His Cys Val Tyr Asp Leu
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<222> 207...215

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Ala Cys Gly His Arg Arg Gly Tyr Ser
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Ser Asp Asn Leu Arg Val Ser Ser Leu
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Lys Arg Leu Gly Asn Asp Ile Ala Leu
5

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<222> 435...443
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Thr Arg Val Thr Ser Phe Leu Asp Trp
5

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Tyr Gly Gly Ile Ile Ser Pro Ser Met
5

<210> 122
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<222> 410...418
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<400> 122

Arg Arg Leu Trp Lys Leu Val Gly Ala
5

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His Arg Arg Gly Tyr Ser Ser Arg Ile
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<222> 109...117
<223> TADG-12 peptide
<400> 124

Val Arg Val Gly Gly Gln Asn Ala Val
5

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Val Arg Glu Gly Cys Ala Ser Gly His
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Tyr Arg Cys Arg Ser Ser Phe Lys Cys
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Gly Val Asp Ser Cys Gln Gly Asp Ser
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Ala Glu Val Asn Lys Pro Gly Val Tyr
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